(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 June 2005 (23.06.2005)

PCT

(10) International Publication Number
WO 2005/056782 A2

(51) International Patent Classification⁷: **C12N 9/00**

(21) International Application Number: PCT/US2004/040438

(22) International Filing Date: 3 December 2004 (03.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/526,764 3 December 2003 (03.12.2003) US

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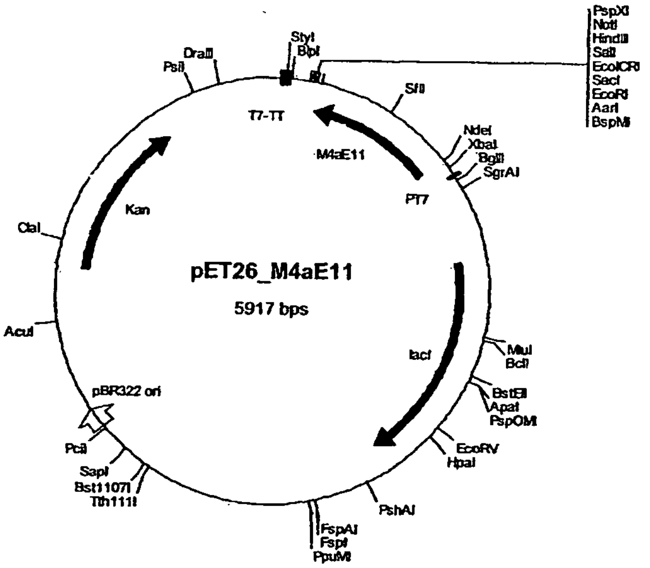
(54) Title: PERHYDROLASE



(57) Abstract: The present invention provides methods and compositions comprising at least one perhydrolase enzyme for cleaning and other applications. In some particularly preferred embodiments, the present invention provides methods and compositions for generation of peracids. The present invention finds particular use in applications involving cleaning, bleaching and disinfecting.

WO 2005/056782 A2

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PERHYDROLASE

The present application claims priority under 35 U.S.C. §119, to co-pending U.S. Provisional Patent Application Serial Number 60/526,764, filed December 3, 2003.

FIELD OF THE INVENTION

The present invention provides methods and compositions comprising at least one perhydrolase enzyme for cleaning and other applications. In some particularly preferred embodiments, the present invention provides methods and compositions for generation of peracids. The present invention finds particular use in applications involving cleaning, bleaching and disinfecting.

BACKGROUND OF THE INVENTION

Detergent and other cleaning compositions typically include a complex combination of active ingredients. For example, most cleaning products include a surfactant system, enzymes for cleaning, bleaching agents, builders, suds suppressors, soil-suspending agents, soil-release agents, optical brighteners, softening agents, dispersants, dye transfer inhibition compounds, abrasives, bactericides, and perfumes. Despite the complexity of current detergents, there are many stains that are difficult to completely remove. Furthermore, there is often residue build-up, which results in discoloration (*e.g.*, yellowing) and diminished aesthetics due to incomplete cleaning. These problems are compounded by the increased use of low (*e.g.*, cold water) wash temperatures and shorter washing cycles. Moreover, many stains are composed of complex mixtures of fibrous material, mainly incorporating carbohydrates and carbohydrate derivatives, fiber, and cell wall components (*e.g.*, plant material, wood, mud/clay based soil, and fruit). These stains present difficult challenges to the formulation and use of cleaning compositions.

In addition, colored garments tend to wear and show appearance losses. A portion of this color loss is due to abrasion in the laundering process, particularly in automated washing and drying machines. Moreover, tensile strength loss of fabric appears to be an unavoidable result of mechanical and chemical action due to use, wearing, and/or washing and drying. Thus, a means to efficiently and effectively wash colored garments so that these appearance losses are minimized is needed.

Cleaning compositions that comprise esterases, lipases and cutinases are well-known in the art. However, these enzymes have a very low ratio of perhydrolysis to hydrolysis. This results in the conversion of most of the ester substrate into acid, instead of the more desirable peracid. This is a serious drawback, since formula space and cost considerations render it feasible to include only a limited amount of substrate.

In sum, despite improvements in the capabilities of cleaning compositions, there remains a need in the art for detergents that remove stains, maintain fabric color and appearance, and prevent dye transfer. In addition, there remains a need for detergent and/or fabric care compositions that provide and/or restore tensile strength, as well as provide anti-wrinkle, anti-bobbling, and/or anti-shrinkage properties to fabrics, as well as provide static control, fabric softness, maintain the desired color appearance, and fabric anti-wear properties and benefits. In particular, there remains a need for the inclusion of compositions that are capable of removing the colored components of stains, which often remain attached to the fabric being laundered. In addition, there remains a need for improved methods and compositions suitable for textile bleaching.

In addition to the fabric and garment cleaning area, bleaching is commonly used in the pulp and paper industry. Prior to production of paper, pulp is typically treated to remove undesirable colored contaminants. This provides pulp that is suitable for production of paper of higher quality than pulp that is not treated to remove colored contaminants and other undesirable components present in pulp. For example, in the paper recycling industry, removal of ink is necessary. Although standard methods are suitable for deinking paper with oil or water-based inks, the increased use of electrostatic inks has made deinking problematic, as these inks are much more difficult to remove. There are various methods available for deinking paper, including the use of enzymes (*See e.g.*, U.S. Patent No. 5,370,770). However, there remains a need in the art for efficient, cost-effective methods for treatment of pulp for paper (recycled and new) product production.

Bleaching is also commonly used in the personal care market (*e.g.*, dental whiteners, hair bleachers, etc.). Although personal care bleaching products have improved over the years, there remains a need for mild, easy to use, cost-effective bleaching methods for this setting.

SUMMARY OF THE INVENTION

The present invention provides methods and compositions comprising at least one perhydrolase enzyme for cleaning and other applications. In some particularly preferred embodiments, the present invention provides methods and compositions for generation of peracids. The present invention finds particular use in applications involving cleaning, bleaching and disinfecting.

In some embodiments, the present invention provides compositions comprising at least one perhydrolase, wherein the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1.

The present invention also provides isolated perhydrolases, wherein the perhydrolases exhibit a perhydrolysis to hydrolysis ratio that is greater than 1. In some preferred embodiments, the perhydrolase is *M. smegmatis* perhydrolase. In alternative preferred embodiments, the perhydrolase is at least approximately about 35% homologous to *M. smegmatis* perhydrolase. In further embodiments, the perhydrolase is at least approximately about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% homologous to *M. smegmatis* perhydrolase. In additional preferred embodiments, the perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2. In some preferred embodiments, the perhydrolases have immunological cross-reactivity with *M. smegmatis* perhydrolase. In still further embodiments, the perhydrolase is at least a portion of *M. smegmatis* perhydrolase, wherein the perhydrolase has a perhydrolysis to hydrolysis ration that is greater than 1. In alternative embodiments, the perhydrolase is a structural homologue of *M. smegmatis* perhydrolase, in which the active site is homologous to at least one amino acid selected from the group consisting of S11, D192, and H195 of the *M. smegmatis* perhydrolase.

The present invention also provides isolated perhydrolase variants having amino acid sequences comprising at least one modification of an amino acid made at a position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2. In some embodiments, at least one modification is made at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein the modified amino acid is selected from the group consisting of Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196. In further embodiments, the modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of M1, K3, R4, I5, L6, C7, D10, S11, L12, T13, W14, W16, G15, V17, P18, V19, D21, G22, A23, P24, T25, E26, R27, F28, A29, P30, D31, V32, R33, W34, T35, G36, L38, Q40, Q41, D45, L42, G43, A44, F46, E47, V48, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, I60, D61, D62, P63, T64, D65, P66, R67, L68, N69, G70, A71, S72, Y73, S76, C77, L78, A79, T80, L82, P83, L84, D85, L86, V87, N94, D95, T96, K97, Y99F100, R101, R102, P104, L105, D106, I107, A108, L109, G110, M111, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P146, P148, W149, F150, I153, F154, I194, and F196.

In some preferred embodiments, the variant perhydrolase exhibits a change in peracid hydrolysis compared to the wild-type perhydrolase. In some embodiments, the change in peracid hydrolysis is a decrease, while in other embodiments, the change in peracid hydrolysis is an increase.

In some alternative preferred embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.1 or less, in comparison with wild-type perhydrolase. In alternative preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of R4, L12, G15, P18, R27, W34L38, A44, E51, G52, L53, S54, T58, R67, L68, S72, A79, T80, D85, L86, V87, N94, K97, R101, V118, L119, G124, G126, and I194.

In further alternative embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.2 or less, in comparison with wild-type perhydrolase. In yet additional embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in

M. smegmatis perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, P18, V19, T25, R27, W34, L38, A44, I49, E50, E51, G52, L53, S54, A55, R56, T58, N59, D62, T64, D65, R67, L68, N69, S72, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, T96, K97, R101, L82, P83, L86, V87, N94, T96, K97, F100, R101, L109, M111, L114, V118, L119, A122, G124, G126, T127, Y129, W149, and I194.

In additional embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.3 or less, in comparison with wild-type perhydrolase. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, L12, P18, V19, G22, A23, T25, E26, R27, W34, G36, L38, Q41, L42, G43, A44, I49, E50, E51, G52, L53, S54, A55, R56, T57, N59, T58, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, T96, K97, Y99, F100, R101, R102, P104, L109, G110, M111, L114, V118, L119, A122, G124, V125, G126, T127, Y129, W149, F154, and I194.

In yet further embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.4 or less, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of R4, I5, L6, D10, S11, L12, W14, G15, W16, P18, V19, G22, A23, T25, E26, R27, F28, W34, T35, G36, L38, Q41, L42, G43, A44, D45, E47, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, T58, I60, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76,

C77, A79, T80, L82, P83, D85, L86, V87, N94, P66, T96, K97, Y99, F100, R101, R102, P104, I107, L109, G110, M111, S112, L114, V118, L119, S121, A122, G124, V125, G126, T127, Y129, W149, F150, F154, I194, and F196.

In some embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.5 or less, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

In additional embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.6 or less, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in

M. smegmatis perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, V125, V19, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

In yet additional embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.7 or less, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119,

L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

In still further embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.8 or less, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119,

L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y1d29, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95, E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89, L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117, R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19, V32, V87, W149, Y129, Y73, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

In additional embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis of about 1.5 or greater, in comparison with wild-type perhydrolase. In some preferred embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95, E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89, L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117,

R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19, V32, V87, W149, Y129, and Y73, Y99, A108, A44, C7, D10, D106, D31, D61, D85, E26, E51, F100, F28, F46, G110, G22, G36, G43, G52, G70, I107, I153, I49, I5, I89, K3, L105, L53, L6, L78, L86, M1, N69, P104, P146, P18, P24, P30, P83, Q117, Q40, Q41, R102, R27, R33, R4, S121, S72, S76, T120, T128, T13, T35, T80, T96, V115, V118, V32V48, V87, W34, G190, V191, G193, T197, E198, A199, R202, D203, G205, V206, A209, E210, Q211, S214, and L215.

In additional embodiments, the variant perhydrolase exhibits a ratio of peracid hydrolysis between about 1.2 and about 1.5, in comparison with wild-type perhydrolase. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, C7, D106, D31, D61, D85, E26, E50, E51, F100, F150, F28, F46, G110, G126, G22, G70, I107, K3, L105, L42, L6, L78, M111, N59, N69, P104, P146, P148, P18, P30, P63, Q117, Q40, Q41, R102, R27, R33, R4, S54, S76, T116, T120, T128, T64, T80, T96, V113, V115, V118, W34, and Y73.

In yet further embodiments, the present invention provides variant perhydrolases in which the variant perhydrolases exhibit a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is at least about 1.2. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of C7, D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41, L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66, R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97, R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124, V125, G126, T127, P148, F150, I153, F154, and F196.

In further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less. In some embodiments, the variant perhydrolase comprising at least one modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A108, A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D45, D61, D62, D65, D85, E26, E47, E50, E51, F100, F150, F154, F196, F28, F46, G110, G124, G126, G15, G22, G36, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105, L109, L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, K86, M1, M111, N59N94, P146, P18, P24, P30, P66, P83, Q40, Q41, R101, R102, R27, R33, R4, R56, R67, S11, S112, S54, S72, S76, T103, T116, T120, T127, T128, T13, T25, T35, T57, T64, T80, T96, V113, V115, V118, V125, V17, V19, V32, V48, V87, W13, W149, W16, W34, Y129, Y73, and Y99.

In alternative embodiments, the present invention provides variant perhydrolases comprising at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A108, A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D31, D45, D61, D62, D65, D85, E26, E47, E50, E51, F100, F150, F154F196, F28, F46, G110, G124, G126, G15, G22, G36, G43, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105, L109, L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, L86, M1, M111, N59, N69, N94, P104, P146, P148, P18, P24, P30, P63, P66, P83, Q117, Q40, Q41, R101, R102, R27, R33, R4, R56, R67, S11, S112, S121, S54, S72, S76, T103, T116, T120, T127, T128, T13, T25, T35, T57, T58, T64, T80, T96,

V113, V115, V118, V125, V17, V19, V32, V48, V87, W14, W149, W16, W34, Y129, Y73, and Y99.

In yet additional embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 1.2 and about 2. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of C7, D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41, L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66, R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97, R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124, V125, G126, T127, P148, F150, I153, F154, F196, G190, E198, A199, R202, D203, V206, A209, E210, Q211, and V212.

In still further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2 and about 2.5. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A44, C7, D10, D85, D95, E26, E47, I107, L12, L42, P104, P148, S54, Q40, Q117, D203, V206, E210.

In still further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2.5 and about 3. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A44, C7, I107, K97, L12, L78, P104, Q40, and V125.

In further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 3.0 and about 5. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of D10, D85, L53, L78, and S54.

In still further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.1 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, and W34.

In further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.2 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, and Y73.

In additional embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.3 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, and Y129.

In yet additional embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.4 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, and V87.

In additional embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.5 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76,

T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, and Y129.

In further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.6 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising t least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149, and Y73.

In yet further embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.7 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149,Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, and Y99.

In additional embodiments, the variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149,Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, Y99, A108, A122, A29, A55, C77, D10, D106, D45, D61, D62, D65, D85,

E47, E50, F100, F150, F28, F46, G110, G124, G126, G15, G36, I153, I194, I5, I60, I89, K3, K97, L105, L109, L114, L119, L38, L42, L68, L84, L86, M1, N59, P24, P30, P83, R101, R27, R4, R56, S112, S54, S76, T103, T116, T120, T127, T128, T13, T35, T64, V113, V17, V19, V32, V48, V87, Y129, Y73, and Y99.

The present invention also provides perhydrolase variants, wherein the perhydrolase variants exhibit greater perhydrolysis activity and decreased peracid hydrolysis activity as compared to wild-type perhydrolase. In some embodiments, the variant perhydrolases exhibit perhydrolysis activity ratio of at least about 1.2, and peracid hydrolysis activity ratio of about 0.8 or less, as compared to wild-type perhydrolase. In alternative embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A29, A44, A55, A71, A79, C7, D10, D106, D31, D85, E26, E47, F150, F154, F196, F28, G124, G126, G36, G43, I153, L109, L42, L53, L109, L42, L53, L109, L42, L53, L68, L82, L86, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S54, S121, S72, S76, T25, T64, V115, and V19.

In additional embodiments, the perhydrolase exhibits perhydrolysis activity ratio of at least about 1.2, a peracid hydrolysis activity ratio of about 0.8 or less, and a protein concentration ratio of at least 0.5, as compared to wild-type perhydrolase. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A29, A44, A71, A79, C7, D85, E26, E47, E51, F150, F154, F196, F28, G124, G126, G36, I153, L109, L12, L53, L68, L82, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S121, S54, S72, S76, T25, T64, V125, and V19.

The present invention provides variant perhydrolases that exhibit an increase in expression of the perhydrolase variants, as compared to the expression of wild-type perhydrolase. In some embodiments, the variant perhydrolase comprises at least one modification comprising at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein at least one substitution is selected from the group consisting of A2, I5, C7, F8, S11, L12, T13, W14, W16, V17, P18, V19, E20, G22, A23, P24, T25, A29, P30, V32, T35, G36, V37, A39, F46, E47, S54, A55, R56, T58, I60, D61, D62, P63, T64, P66, R67, L68, N69, G70, S72, Y73, L74, P75, S76, C77, L78, A79, T80, L82, P83, L84, L86, I89, T93, T96, K97, A98, Y99, F100, R101, R102, T103, P104, L105, D106, I107, A108, L109, G110, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P130, P132, K133, L135, V136, S138, P141, L142, A143, M145, H147, W149, F150, Q151, I153, G157, Q159, T161, T162, L164, A165, R166, V167, Y168, A170, L171, A172, M175, K176, P178, A182, G183, S184, V185, I186, T188, I194, F196, V191, N201, L208, A209, Q211, Q213, S214, L215, and L216.

The present invention also provides isolated proteins comprising homologs of *M. smegmatis* perhydrolase, wherein the homologs are proteins within the SGNH-hydrolase family of proteins. In alternative preferred embodiments, the isolated proteins have at least about 35% identity with the amino acid sequence of *M. smegmatis* perhydrolase, in which the protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195. In further embodiments, the perhydrolase is at least approximately about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% homologous to *M. smegmatis* perhydrolase. In additional preferred embodiments, the perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2.

The present invention also provides isolated proteins having at least about 38% identity with the amino acid sequence of *M. smegmatis* perhydrolase, wherein the protein exhibits perhydrolysis activity. In further embodiments, the perhydrolase is at least approximately about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% homologous to *M. smegmatis* perhydrolase. In additional preferred embodiments, the perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2.

The present invention also provides homologs of *M. smegmatis* perhydrolase, wherein the homologs are perhydrolases comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT. In preferred embodiments, the homologs exhibit perhydrolysis. In some particularly preferred embodiments, the homologs exhibit a perhydrolysis to hydrolysis ratio that is great than about 1. In still further embodiments, the homologs are immunologically cross-reactive with antibodies raised against *M. smegmatis* perhydrolase. In yet additional embodiments, antibodies raised against the homolog cross-react with *M. smegmatis* perhydrolase.

The present invention also provides isolated proteins having at least-about 35% identity with the amino acid sequence of at least one *M. smegmatis* perhydrolase homolog, wherein the proteins exhibit perhydrolysis activity.

In some particularly preferred embodiments, the present invention provides proteins having perhydrolase activity, wherein the proteins are in the form of a multimer in solution. In some more preferred embodiments, the protein is a perhydrolase that comprises a dimer. In alternative particularly preferred embodiments, the protein is a perhydrolase that comprises an octamer. In still further embodiments, the protein is in the form of a multimer in solution and the protein is selected from the group consisting of *M. smegmatis* perhydrolase, *M. smegmatis* perhydrolase homologs, and *M. smegmatis* perhydrolase variants. In yet further embodiments, the protein is selected from the group consisting of modified serine hydrolases and modified cysteine hydrolases, wherein the modified serine hydrolases or modified cysteine hydrolases comprise increased perhydrolase activity as compared to unmodified serine hydrolases or unmodified cysteine hydrolases

The present invention also provides proteins having perhydrolase activity, wherein the protein comprises at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT. In some embodiments, the protein is obtained from a member of the *Rhizobiales*. In some preferred embodiments, the protein is obtained from a member of the genus *Mycobacterium.*

The present invention also provides isolated genes identified using at least one primer selected from the group consisting of SEQ ID NOS:21-69.

The present invention also provides methods for identifying a perhydrolase, comprising the steps of: identifying source of the perhydrolase; analyzing the source to identify sequences comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT; expressing the sequences identified in step b) to produce the perhydrolase; and testing the perhydrolase for perhydrolysis activity.

In some embodiments, the analyzing step is an amplification step wherein the primer sequences set forth in SEQ ID NOS:21-69 are used to amplifying the sequences comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT. In still further embodiments, the source is selected from the group consisting of environmental sources and metagenomic sources. The present invention also provides proteins identified using the methods set forth herein. The present invention further provides isolated nucleic acid sequences encoding the proteins identified using the methods set forth herein. In some particularly preferred embodiments, the proteins exhibit a perhydrolysis to hydrolysis ratio that is greater than about 1. In still further embodiments, the proteins exhibit a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase. In yet additional embodiments, the proteins comprise at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195.

In further embodiments, the analyzing step comprises searching at least one amino acid database. In yet further embodiments, the analyzing step comprises searching at least one nucleic acid database to identify nucleic acid sequences encoding the amino acid sequences of the perhydrolase. In still further embodiments, the source is selected from the group consisting of environmental sources and metagenomic sources. The present invention further provides isolated nucleic acid sequences encoding the proteins identified using the methods set forth herein. In some particularly preferred embodiments, the proteins exhibit a perhydrolysis to hydrolysis ratio that is greater than about 1. In still further embodiments, the proteins exhibit a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase. In yet additional embodiments, the proteins comprise at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195, as set forth in SEQ ID NO:2.

The present invention also provides variant perhydrolases having altered substrate specificities as compared to wild-type *M. smegmatis* perhydrolase. In some embodiments, the variant perhydrolases have altered para nitrophenyl caproate (PNC) activity, as compared to wild-type *M. smegmatis* perhydrolase.

The present invention also provides variant perhydrolases having altered pI values as compared to wild-type *M. smegmatis* perhydrolase. In some embodiments, the variant perhydrolases comprise at least one positively charged mutation, while in alternative embodiments, the variant perhydrolases comprise at least one negatively charged mutation.

The present invention also provides variant perhydrolases that have increased stability, as compared to wild-type *M. smegmatis* perhydrolase. In some preferred embodiments, the stability of the variant perhydrolase is selected from the group consisting of thermostability, enzymatic stability, and chemical stability.

The present invention also provides variant perhydrolases, wherein the variant perhydrolase exhibits at least one altered surface property. In some preferred embodiments, the variants comprise at least one mutation comprising at least one substitution at sites selected from the group consisting of the residues set forth in Table 15-1.

The present invention also provides perhydrolase variants having at least one improved property as compared to wild-type perhydrolase.

The present invention also provides expression vectors comprising a polynucleotide sequence encoding at least one perhydrolase variant. The present invention further provides host cells comprising at least one such expression vector. In some preferred embodiments, a host cell is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp. The present invention also provides perhydrolases produced by the host cells.

The present invention also provides compositions comprising at least a portion of at least one perhydrolase. In some preferred embodiments, the perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2. In further embodiments, the perhydrolase is encoded by a polynucleotide sequence comprises SEQ ID NO:1. In additional embodiments, the sequence comprises at least a portion of SEQ ID NO:1. In further embodiments, the present invention provides expression vectors comprising the polynucleotide sequence encoding at least a portion of at least one perhydrolase. The present invention also provides host comprising at least one expression vectors. In some embodiments, the host cells are selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp. The present invention also provides perhydrolases produced by these host cells.

The present invention also provides variant perhydrolases, wherein the perhydrolases comprise at least one substitution corresponding to the amino acid positions in SEQ ID NO:2, and wherein the variant perhydrolase has better performance in at least one property, compared to wild-type *M. smegmatis* perhydrolase.

The present invention further provides isolated polynucleotides comprising a nucleotide sequence (i) having at least about 70% identity to SEQ ID NO:1, or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence set forth in SEQ ID NO:1, under conditions of intermediate to high stringency, or (iii) being complementary to the nucleotide sequence set forth in SEQ ID NO:1. In some embodiments, the present invention also provides vectors comprising these polynucleotide sequences. In additional embodiments, the present invention also provides host comprising at least one expression vectors. In some embodiments, the host cells are selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp. The present invention also provides perhydrolases produced by these host cells.

The present invention also provides polynucleotides comprising a sequence complementary to at least a portion of the sequence set forth in SEQ ID NO:1.

The present invention also provides methods of producing enzymes having perhydrolase activity, comprising: transforming a host cell with an expression vector comprising a polynucleotide having at least 70% sequence identity to SEQ ID NO:1; cultivating the transformed host cell under conditions suitable for the host cell to produce the perhydrolase; and recovering the perhydrolase. In some preferred embodiments, the host cell is selected from the group consisting of *Streptomyces*, *Pantoea*, *Escherichia*, and *Bacillus* species.

The present invention also provides probes comprising a 4 to 150 polynucleotide sequence substantially identical to a corresponding fragment of SEQ ID NO:1, wherein the probe is used to detect a nucleic acid sequence coding for an enzyme having perhydrolase activity.

The present invention also provides cleaning compositions comprising: a) at least 0.0001 weight percent of a perhydrolase that exhibits a perhydrolysis to hydrolysis ratio that is greater than 1; b) a molecule comprising an ester moiety; and c) optionally, an adjunct ingredient.

The present invention further provides cleaning compositions comprising: a) at least 0.0001 weight percent of a perhydrolase that exhibits a perhydrolysis to hydrolysis ratio that is greater than 1; b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, the peroxygen source being selected from the group consisting of: a per-salt; an organic peroxyacid; urea hydrogen peroxide; a carbohydrate and carbohydrate oxidase mixture, and mixtures thereof; c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety; and d) optionally, an adjunct ingredient.

The present invention also provides cleaning compositions comprising: a) from about 0.0001 to about 1 weight percent of a variant perhydrolase having an amino acid sequence comprising at least one modification of an amino acid made at a position equivalent to a position in *M smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2; b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, the peroxygen source being selected from the group consisting of: a per-salt; an organic peroxyacid; urea hydrogen peroxide; a carbohydrate and carbohydrate oxidase mixture; and mixtures thereof; c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety; and d) optionally, an adjunct ingredient. In some preferred embodiments, the cleaning compositions further comprise at least one adjunct ingredient. In some particularly preferred embodiments, the adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids, pigments and mixtures thereof.

In additional embodiments, the present invention provides cleaning compositions wherein: the perhydrolase exhibits a perhydrolysis to hydrolysis molar ratio that is greater than about 0.1; the per-salt is selected from the group consisting of alkalimetal perborate, alkalimetal percarbonate, alkalimetal perphosphates, alkalimetal persulphates and mixtures thereof; the carbohydrate is selected from the group consisting of mono-carbohydrates, di- carbohydrates, tri- carbohydrates, oligo- carbohydrates and mixtures thereof; the carbohydrate oxidase is selected from the group consisting of aldose oxidase (IUPAC classification EC1.1.3.9), galactose oxidase (IUPAC classification EC1.1.3.9), cellobiose oxidase (IUPAC classification EC1.1.3.25), pyranose oxidase (IUPAC classification EC1.1.3.10), sorbose oxidase (IUPAC classification EC1.1.3.11) hexose oxidase (IUPAC classification EC1.1.3.5). glucose oxidase (IUPAC classification EC1.1.3.4) and mixtures thereof; and the molecule comprising an ester moiety has the formula:

$$R^1O_x[(R^2)_m(R^3)_n]_p$$

(i) wherein R^1 is a moiety selected from the group consisting of H, substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;

(ii) each R^2 is an alkoxylate moiety;

(iii) R^3 is an ester-forming moiety having the formula:

R^4CO- wherein R^4 is H, alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;

(iv) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1;

(v) p is an integer that is equal to or less than x;

(vi) m is an integer from 0 to 50; and

(vii) n is at least 1

In alternative embodiments, the present invention provides cleaning compositions wherein: a) R^1 is an C_2-C_{32} substituted or unsubstituted alkyl or heteroalkyl moiety; b) each R^2 is independently an ethoxylate or propoxylate moiety; and c) m is an integer from 1 to 12. In some embodiments, R^3 is an ester-forming moiety having the formula: R^4CO- wherein R^4 is: a) a substituted or unsubstituted alkyl, alkenyl or alkynyl moiety comprising from 1 to 22 carbon atoms; or b) a substituted or unsubstituted aryl, alkylaryl, alkylheteroaryl or heteroaryl moiety comprising from 4 to 22 carbon atoms.

In still further embodiments of the cleaning compositions, the molecule comprising the ester moiety has the formula:

$$R^1O_x[(R^2)_m(R^3)_n]_p$$

wherein: a) R^1 is H or a moiety that comprises a primary, secondary, tertiary or quaternary amine moiety, the R^1 moiety that comprises an amine moiety being selected from the group consisting of substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl; b) each R^2 is an alkoxylate moiety; c) R^3 is an ester-forming moiety having the formula: R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl; d) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1; e) p is an integer that is equal to or less than x; f) m is an integer from 0 to 12; and g) n is at least 1.

In still further embodiments of the present cleaning compositions, the molecule comprising an ester moiety has a weight average molecular weight of less than 600,000 Daltons. In yet additional embodiments, an adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids, pigments and mixtures thereof.

The present invention further provides methods of cleaning comprising the steps of: a) contacting a surface and/or an article comprising a fabric with any of the cleaning compositions provided above and/or a composition comprising any of the cleaning compositions provided above; and b) optionally washing and/or rinsing the surface or material.

In alternative embodiments, the present invention provides methods of cleaning, the method comprising the steps of: a) contacting a surface and/or an article comprising a fabric with any suitable cleaning composition provided above and/or a composition comprising any suitable cleaning provided above; and b) optionally washing and/or rinsing the surface or material.

The present invention also provides bleaching compositions comprising at least one perhydrolase. In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides bleaching compositions comprising at least one perhydrolase variant having an amino acid sequence comprising at least one modification of an amino acid made at a position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides bleaching compositions comprising at least one perhydrolase variant having at least one improved property as compared to wild-type perhydrolase. In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases,. mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides bleaching compositions comprising at least one perhydrolase variant comprising at least one substitution corresponding to the amino acid positions in SEQ ID NO:2, and wherein the variant perhydrolase has better performance in at least one property compared to wild-type *M. smegmatis* perhydrolase. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides bleaching compositions comprising at least one perhydrolase that is at least approximately about 35% homologous to *M. smegmatis* perhydrolase. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides disinfecting compositions comprising at least one perhydrolase. In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides disinfecting compositions comprising at least one perhydrolase variant having an amino acid sequence comprising at least one modification of an amino acid made at a position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides disinfecting compositions comprising at least one perhydrolase variant having at least one improved property as compared to wild-type perhydrolase. In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides disinfecting compositions comprising at least one perhydrolase variant comprising at least one substitution corresponding to the amino acid positions in SEQ ID NO:2, and wherein the variant perhydrolase has better performance in at least one property compared to wild-type *M. smegmatis* perhydrolase. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

The present invention also provides disinfecting compositions comprising at least one perhydrolase that is at least approximately about 35% homologous to *M. smegmatis* perhydrolase. . In some particularly preferred embodiments, the perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1. In some embodiments, the bleaching compositions further comprise at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

In some preferred embodiments, the perhydrolase is at least approximately 70% homologous to *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2. In some embodiments, the present invention provides perhydrolases that cross react with antibody generated against *M. smegmatis* perhydrolase, particularly that comprising the amino acid sequence set forth in SEQ ID NO:2. In further embodiments, the present invention provides perhydrolases that are structural homologs of the *M. smegmatis* perhydrolase, in which active site comprises sites homologous to S11, D192, and H195 of the *M. smegmatis* perhydrolase. In yet additional embodiments, the present invention provides perhydrolases comprising one or more modifications at the following residues: Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196. However, it is not intended that the present invention be limited to perhydrolases with these modifications only at these residues, as perhydrolases with other modifications also find use with the present invention.

In some embodiments, at least one perhydrolase of the present invention is used in a cleaning process wherein an article to be cleaned is exposed to a sufficient amount of the at least one perhydrolase under conditions such that the perhydrolase cleans and/or bleaches, and/or decolorizes any/all stains present on the article (*e.g.*, laundry and dish detergents). In some embodiments, the cleaning further comprises disinfecting. In some embodiments, the article cleaned, bleached and/or disinfected using at least one perhydrolase of the present invention comprises textiles and/or hard surfaces, while in other embodiments, the article is paper or pulp, and in still further embodiments, at least one perhydrolase is used as a personal care product to whiten or bleach hair, teeth, skin, etc. Thus, in some embodiments, the present invention provides compositions for use in various cleaning, bleaching, and/or disinfecting applications. Indeed, it is not intended that the present invention be limited to any particular application.

In some preferred embodiments, the perhydrolase comprises SEQ ID NO:2. In some preferred alternative embodiments, the perhydrolase is encoded by the nucleic acid sequence set forth in SEQ ID NO:1.

In some embodiments, the present invention provides enzymes with activities that result in high peracid/acid ratios. In alternative embodiments, the present invention provides the perhydrolase of *Mycobacterium smegmatis*, as well as sequence and/or structural homologs of this protein. In additional embodiments, the present invention provides enzymes that have been modified so as to express perhydrolase activity with a high perhydrolysis to hydrolase ratio either in addition to or instead of the enzyme's original activity. In additional embodiments, the present invention provides modified enzymes with altered substrate specificity, Km, kcat, perhydrolase activity, and/or peracid degradation activity.

In additional embodiments, the present invention provides means to identify, produce, and characterize enzymes that comprise the perhydrolysis activity of the present invention. The present invention further provides methods and compositions comprising at least one perhydrolase for cleaning, disinfecting, bleaching, and other applications, including but not limited to paper and pulp bleaching, fabric and garment cleaning, hard surface cleaning, and personal care applications (*e.g.*, oral care, hair care, and skin care). In some preferred embodiments, the present invention provides methods and compositions for bleaching cotton and other fabrics. Indeed, the present invention finds use in the bleaching and cleaning of various textiles. It is not intended that the present invention be limited to any particular setting, application or use, as it is contemplated that it will find use in numerous areas where an enzymatic generation of peracids is desired over the use of preformed peracids or hydrogen peroxide or other bleaching chemicals, under conditions including but not limited to a wide range of pHs and temperatures. The present invention also finds use in applications where peracid hydrolysis is useful, such as in the clean up of peracids.

Furthermore, the present invention provides means to produce perhydrolase enzymes suitable for cleaning, disinfecting, bleaching, and other applications, including personal care.

DESCRIPTION OF THE FIGURES

Figure 1 provides a phylogenetic tree of *M. smegmatis* perhydrolase and other related sequences.

Figure 2 provides an overview phylogenetic tree, showing the major branches of the bacteria and the origin of the active clones/sequences compared to *M. smegmatis*.

Figure 3 provides a schematic of four structural families of serine hydrolases, including perhydrolase (SGNH-hydrolase family), chymotrypsin, subtilisin, and α/β hydrolase.

Figure 4 provides a diagram of the structure of the perhydrolase fold.

Figure 5 provides a map of plasmid pET26-M4aE11.

Figure 6 provides a purification table showing the enzyme activity of the enzyme of the present invention through various steps in the purification process.

Figure 7 provides a graph which shows the ratio of perbutyric acid to butyric acid generated by various enzymes from 10 mM tributyrin and 29 mM hydrogen peroxide in 40 minutes.

Figure 8 provides a graph showing the peracid production by 30 mM acetate equivalents and 29 mM hydrogen peroxide, tested at various pHs. These results show that using the perhydrolase composition of the present invention, there is peracid generation over a wide pH range. In contrast, with TAED and hydrogen peroxide, peracid generation is limited to alkaline conditions.

Figure 9 provides a graph showing the peracid production by 0.1 ppm perhydrolase enzyme in 30 mM ethyl acetate and 20 mM hydrogen peroxide at various temperatures. These results show that the perhydrolase of the present invention works at a wide range of temperatures, including low temperatures.

Figure 10 provides a graph showing the ratio of perbutyric acid to butyric acid generated by various enzymes from 10 mM tributyrin and 29 mM hydrogen peroxide in 4, 10, and 30 minutes.

Figure 11 provides a graph showing the ratio of peracetic acid to acetic acid generated by various enzymes from 10 mM triacetin and 29 mM hydrogen peroxide in 4 and 10 minutes.

Figure 12 provides a map of plasmid pMSATNcoI.

Figure 13 provides a map of plasmid pMSATNco1-1.

Figure 14 provides a map of plasmid pAH505.

Figure 15 provides a map of plasmid pSFNASally.

Figure 16 provides a map of plasmid pCP606.

Figure 17 provides a map of plasmid pCP649.

Figure 18 provides a map of plasmid pSECGT-MSAT.

Figure 19 provides a map of plasmid pSEGT-phdA4.

Figure 20 provides a map of plasmid pMC355rbs.

Figure 21 provides a graph showing the degree of bleaching by three detergents tested alone and in comparison with the *M. smegmatis* perhydrolase of the present invention.

Figure 22 provides a graph showing the bleaching ability of the *M. smegmatis* perhydrolase tested on cotton.

Figure 23 provides a graph showing the bleaching ability of the *M. smegmatis* perhydrolase tested on linen.

DESCRIPTION OF THE INVENTION

The present invention provides methods and compositions comprising at least one perhydrolase enzyme for cleaning and other applications. In some particularly preferred embodiments, the present invention provides methods and compositions for generation of peracids. In particular, the present invention provides improved methods and compositions comprising perhydrolysis enzymes with high peracid/acid ratios for cleaning, bleaching, disinfecting and other applications. In some preferred embodiments, the present invention provides improved methods and compositions for generation of peracids. The present invention finds particular use in applications involving cleaning, bleaching and disinfecting.

Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, microbiology, protein purification, protein engineering, protein and DNA sequencing, and recombinant DNA fields, which are within the skill of the art. Such techniques are known to those of skill in the art and are described in numerous texts and reference works (*See e.g.*, Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual", Second Edition (Cold Spring Harbor), [1989]); and Ausubel *et al.*, "Current Protocols in Molecular Biology" [1987]). All patents, patent applications, articles and publications mentioned herein, both *supra* and *infra*, are hereby expressly incorporated herein by reference.

Furthermore, the headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole. Nonetheless, in order to facilitate understanding of the invention, a number of terms are defined below.

Definitions

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology*, 2d Ed., John Wiley and Sons, NY (1994); and Hale and Marham, *The Harper Collins Dictionary of Biology*, Harper Perennial, NY (1991) provide those of skill in the art with a general dictionaries of many of the terms used in the invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole. Also, as used herein, the singular terms "a", "an," and "the" include the plural reference unless the context clearly indicates otherwise. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.

It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

As used herein, the term "bleaching" refers to the treatment of a material (*e.g.*, fabric, laundry, pulp, etc.) or surface for a sufficient length of time and under appropriate pH and temperature conditions to effect a brightening (*i.e.*, whitening) and/or cleaning of the material. Examples of chemicals suitable for bleaching include but are not limited to ClO_2, H_2O_2, peracids, NO_2, etc.

As used herein, the term "disinfecting" refers to the removal of contaminants from the surfaces, as well as the inhibition or killing of microbes on the surfaces of items. It is not intended that the present invention be limited to any particular surface, item, or contaminant(s) or microbes to be removed.

As used herein, the term "perhydrolase" refers to an enzyme that is capable of catalyzing a reaction that results in the formation of sufficiently high amounts of peracid suitable for applications such as cleaning, bleaching, and disinfecting. In particularly preferred embodiments, the perhydrolase enzymes of the present invention produce very high perhydrolysis to hydrolysis ratios. The high perhydrolysis to hydrolysis ratios of these distinct enzymes makes these enzymes suitable for use in a very wide variety of applications. In additional preferred embodiments, the perhydrolases of the present invention are characterized by having distinct tertiary structure and primary sequence. In particularly preferred embodiments, the perhydrolases of the present invention comprises distinct primary and tertiary structures. In some particularly preferred embodiments, the perhydrolases of the present invention comprise distinct quaternary structure. In some preferred embodiments, the perhydrolase of the present invention is the *M. smegmatis* perhydrolase, while in alternative embodiments, the perhydrolase is a variant of this perhydrolase, while in still further embodiments, the perhydrolase is a homolog of this perhydrolase. In further preferred embodiments, a monomeric hydrolase is engineered to produce a multimeric enzyme that has better perhydrolase activity than the monomer. However, it is not intended that the present invention be limited to this specific *M. smegmatis* perhydrolase, specific variants of this perhydrolase, nor specific homologs of this perhydrolase.

As used herein, the term "multimer" refers to two or more proteins or peptides that are covalently or non-covalently associated and exist as a complex in solution. A "dimer" is a multimer that contains two proteins or peptides; a "trimer" contains three proteins or peptides, etc. As used herein, "octamer" refers to a multimer of eight proteins or peptides.

As used herein, the phrase "perhydrolysis to hydrolysis ratio" is the ratio of the amount of enzymatically produced peracid to that of enzymatically produced acid by the perhydrolase, under defined conditions and within a defined time. In some preferred embodiments, the assays provided herein are used to determine the amounts of peracid and acid produced by the enzyme.

As used herein, "personal care products" means products used in the cleaning, bleaching and/or disinfecting of hair, skin, scalp, and teeth, including, but not limited to shampoos, body lotions, shower gels, topical moisturizers, toothpaste, and/or other topical cleansers. In some particularly preferred embodiments, these products are utilized on humans, while in other embodiments, these products find use with non-human animals (*e.g.*, in veterinary applications).

As used herein, "pharmaceutically-acceptable" means that drugs, medicaments and/or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and other animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

As used herein, “cleaning compositions” and “cleaning formulations” refer to compositions that find use in the removal of undesired compounds from items to be cleaned, such as fabric, dishes, contact lenses, other solid substrates, hair (shampoos), skin (soaps and creams), teeth (mouthwashes, toothpastes) etc. The term encompasses any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (*e.g.*, liquid, gel, granule, or spray composition), as long as the composition is compatible with the perhydrolase and other enzyme(s) used in the composition. The specific selection of cleaning composition materials are readily made by considering the surface, item or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use.

The terms further refer to any composition that is suited for cleaning, bleaching, disinfecting, and/or sterilizing any object and/or surface. It is intended that the terms include, but are not limited to detergent compositions (*e.g.*, liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile and laundry pre-spotters, as well as dish detergents).

Indeed, the term “cleaning composition” as used herein, includes unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, gel or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) types; liquid fine-fabric detergents; hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type;

machine dishwashing agents, including the various tablet, granular, liquid and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, mouthwashes, denture cleaners, car or carpet shampoos, bathroom cleaners; hair shampoos and hair-rinses; shower gels and foam baths and metal cleaners; as well as cleaning auxiliaries such as bleach additives and "stain-stick" or pre-treat types.

As used herein, the terms "detergent composition" and "detergent formulation" are used in reference to mixtures which are intended for use in a wash medium for the cleaning of soiled objects. In some preferred embodiments, the term is used in reference to laundering fabrics and/or garments (*e.g.*, "laundry detergents"). In alternative embodiments, the term refers to other detergents, such as those used to clean dishes, cutlery, etc. (*e.g.*, "dishwashing detergents"). It is not intended that the present invention be limited to any particular detergent formulation or composition. Indeed, it is intended that in addition to perhydrolase, the term encompasses detergents that contain surfactants, transferase(s), hydrolytic enzymes, oxido reductases, builders, bleaching agents, bleach activators, bluing agents and fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and solubilizers.

As used herein, "enhanced performance" in a detergent is defined as increasing cleaning of bleach-sensitive stains (*e.g.*, grass, tea, wine, blood, dingy, etc.), as determined by usual evaluation after a standard wash cycle. In particular embodiments, the perhydrolase of the present invention provides enhanced performance in the oxidation and removal of colored stains and soils. In further embodiments, the perhydrolase of the present invention provides enhanced performance in the removal and/or decolorization of stains. In yet additional embodiments, the perhydrolase of the present invention provides enhanced performance in the removal of lipid-based stains and soils. In still further embodiments, the perhydrolase of the present invention provides enhanced performance in removing soils and stains from dishes and other items.

As used herein the term "hard surface cleaning composition," refers to detergent compositions for cleaning hard surfaces such as floors, walls, tile, bath and kitchen fixtures, and the like. Such compositions are provided in any form, including but not limited to solids, liquids, emulsions, etc.

As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to granular and liquid forms.

As used herein, "fabric cleaning composition" refers to all forms of detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms.

As used herein, "textile" refers to woven fabrics, as well as staple fibers and filaments suitable for conversion to or use as yarns, woven, knit, and non-woven fabrics. The term encompasses yarns made from natural, as well as synthetic (*e.g.*, manufactured) fibers.

As used herein, "textile materials" is a general term for fibers, yarn intermediates, yarn, fabrics, and products made from fabrics (*e.g.*, garments and other articles).

As used herein, "fabric" encompasses any textile material. Thus, it is intended that the term encompass garments, as well as fabrics, yarns, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material.

As used herein, the term "compatible," means that the cleaning composition materials do not reduce the enzymatic activity of the perhydrolase to such an extent that the perhydrolase is not effective as desired during normal use situations. Specific cleaning composition materials are exemplified in detail hereinafter.

As used herein, "effective amount of perhydrolase enzyme" refers to the quantity of perhydrolase enzyme necessary to achieve the enzymatic activity required in the specific application (*e.g.*, personal care product, cleaning composition, etc.). Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (*e.g.*, granular, bar) composition is required, and the like.

As used herein, "non-fabric cleaning compositions" encompass hard surface cleaning compositions, dishwashing compositions, personal care cleaning compositions (*e.g.*, oral cleaning compositions, denture cleaning compositions, personal cleansing compositions, etc.), and compositions suitable for use in the pulp and paper industry.

As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Oral care compositions that find use in conjunction with the perhydrolases of the present invention are well known in the art (*See e.g.*, U.S. Patent Nos 5,601,750, 6,379,653, and 5,989,526, all of which are incorporated herein by reference).

As used herein, "pulp treatment compositions" refers to the use of the present perhydrolase enzymes in compositions suitable for use in papermaking. It is intended that the term encompass compositions suitable for the treatment of any pulp material, including wood, as well as non-wood materials, such as "agricultural residues" and "fiber crops," including but not limited to wheat straw, rice straw, corn stalks, bagasse (sugar cane), rye grass straw, seed flax straw, flax straw, kenaf, industrial hemp, sisal, textile flat straw, hesperaloe, etc. Thus, the present invention also encompasses the use of the perhydrolases of the present invention in pulp treatment methods.

As used herein, "oxidizing chemical" refers to a chemical that has the capability of bleaching pulp or any other material. The oxidizing chemical is present at an amount, pH and temperature suitable for bleaching. The term includes, but is not limited to hydrogen peroxide and peracids.

As used herein, "acyl" is the general name for organic acid groups, which are the residues of carboxylic acids after removal of the -OH group (*e.g.*, ethanoyl chloride,

$CH_3CO\text{-}Cl$, is the acyl chloride formed from ethanoic acid, $CH_3COO\text{-}H$). The names of the individual acyl groups are formed by replacing the "-ic" of the acid by "-yl."

As used herein, the term "acylation" refers to the chemical transformation which substitutes the acyl (RCO-) group into a molecule, generally for an active hydrogen of an -OH group.

As used herein, the term "transferase" refers to an enzyme that catalyzes the transfer of functional compounds to a range of substrates.

As used herein, "leaving group" refers to the nucleophile which is cleaved from the acyl donor upon substitution by another nucleophile.

As used herein, the term "enzymatic conversion" refers to the modification of a substrate to an intermediate or the modification of an intermediate to an end-product by contacting the substrate or intermediate with an enzyme. In some embodiments, contact is made by directly exposing the substrate or intermediate to the appropriate enzyme. In other embodiments, contacting comprises exposing the substrate or intermediate to an organism that expresses and/or excretes the enzyme, and/or metabolizes the desired substrate and/or intermediate to the desired intermediate and/or end-product, respectively.

As used herein, the phrase "detergent stability" refers to the stability of a detergent composition. In some embodiments, the stability is assessed during the use of the detergent, while in other embodiments, the term refers to the stability of a detergent composition during storage.

As used herein, the phrase, "stability to proteolysis" refers to the ability of a protein (*e.g.*, an enzyme) to withstand proteolysis. It is not intended that the term be limited to the use of any particular protease to assess the stability of a protein.

As used herein, "oxidative stability" refers to the ability of a protein to function under oxidative conditions. In particular, the term refers to the ability of a protein to function in the presence of various concentrations of H_2O_2 and/or peracid. Stability under various oxidative conditions can be measured either by standard procedures known to those in the art and/or by the methods described herein. A substantial change in oxidative stability is evidenced by at least about a 5% or greater increase or decrease (in most embodiments, it is preferably an increase) in the half-life of the enzymatic activity, as compared to the enzymatic activity present in the absence of oxidative compounds.

As used herein, "pH stability" refers to the ability of a protein to function at a particular pH. In general, most enzymes have a finite pH range at which they will function. In addition to enzymes that function in mid-range pHs (*i.e.*, around pH 7), there are enzymes that are capable of working under conditions with very high or very low pHs. Stability at various pHs can be measured either by standard procedures known to those in the art and/or by the methods described herein. A substantial change in pH stability is evidenced by at least about 5% or greater increase or decrease (in most embodiments, it is preferably an increase) in the half-life of the enzymatic activity, as compared to the enzymatic activity at the enzyme's optimum pH. However, it is not intended that the present invention be limited to any pH stability level nor pH range.

As used herein, "thermal stability" refers to the ability of a protein to function at a particular temperature. In general, most enzymes have a finite range of temperatures at which they will function. In addition to enzymes that work in mid-range temperatures (*e.g.*, room temperature), there are enzymes that are capable of working in very high or very low temperatures. Thermal stability can be measured either by known procedures or by the methods described herein. A substantial change in thermal stability is evidenced by at least about 5% or greater increase or decrease (in most embodiments, it is preferably an increase) in the half-life of the catalytic activity of a mutant when exposed to a different temperature (*i.e.*, higher or lower) than optimum temperature for enzymatic activity. However, it is not intended that the present invention be limited to any temperature stability level nor temperature range.

As used herein, the term "chemical stability" refers to the stability of a protein (*e.g.*, an enzyme) towards chemicals that adversely affect its activity. In some embodiments, such chemicals include, but are not limited to hydrogen peroxide, peracids, anionic detergents, cationic detergents, non-ionic detergents, chelants, etc. However, it is not intended that the present invention be limited to any particular chemical stability level nor range of chemical stability.

As used herein, the phrase "perhydrolase activity improvement" refers to the relative improvement of perhydrolase activity, in comparison with a standard enzyme. In some embodiments, the term refers to an improved rate of perhydrolysis product, while in other embodiments, the term encompasses perhydrolase compositions that produce less hydrolysis product. In additional embodiments, the term refers to perhydrolase compositions with altered substrate specificity.

As used herein, the phrase "alteration in substrate specificity" refers to changes in the substrate specificity of an enzyme. In some embodiments, a change in substrate specificity is defined as a difference between the K_{cat}/K_m ratio observed with an enzyme compared to enzyme variants or other enzyme compositions. Enzyme substrate specificities vary, depending upon the substrate tested. The substrate specificity of an enzyme is determined by comparing the catalytic efficiencies it exhibits with different substrates. These determinations find particular use in assessing the efficiency of mutant enzymes, as it is generally desired to produce variant enzymes that exhibit greater ratios for particular substrates of interest. For example, the perhydrolase enzymes of the present invention are more efficient in producing peracid from an ester substrate than enzymes currently being used in cleaning, bleaching and disinfecting applications. Another example of the present invention is a perhydrolase with a lower activity on peracid degradation compared to the wild type. Another example of the present invention is a perhydrolase with higher activity on more hydrophobic acyl groups than acetic acid. However, it is not intended that the present invention be limited to any particular substrate composition nor any specific substrate specificity.

As used herein, "surface property" is used in reference to an electrostatic charge, as well as properties such as the hydrophobicity and/or hydrophilicity exhibited by the surface of a protein.

As used herein, the phrase "is independently selected from the group consisting of . . . ." means that moieties or elements that are selected from the referenced *Markush* group can be the same, can be different or any mixture of elements as indicated in the following example:

A molecule having 3 R groups wherein each R group is independently selected from the group consisting of A, B and C. Here the three R groups may be: AAA, BBB, CCC, AAB, AAC, BBA, BBC, CCA, CCB, or ABC.

In reference to chemical compositions, the term "substituted" as used herein, means that the organic composition or radical to which the term is applied is:

(a) made unsaturated by the elimination of at least one element or radical; or

(b) at least one hydrogen in the compound or radical is replaced with a moiety containing one or more (i) carbon, (ii) oxygen, (iii) sulfur, (iv) nitrogen or (v) halogen atoms; or

(c) both (a) and (b).

Moieties which may replace hydrogen as described in (b) immediately above, that contain only carbon and hydrogen atoms, are hydrocarbon moieties including, but not limited to, alkyl, alkenyl, alkynyl, alkyldienyl, cycloalkyl, phenyl, alkyl phenyl, naphthyl, anthryl, phenanthryl, fluoryl, steroid groups, and combinations of these groups with each other and with polyvalent hydrocarbon groups such as alkylene, alkylidene and alkylidyne groups. Moieties containing oxygen atoms that may replace hydrogen as described in (b) immediately above include, but are not limited to, hydroxy, acyl or keto, ether, epoxy, carboxy, and ester containing groups. Moieties containing sulfur atoms that may replace hydrogen as described in (b) immediately above include, but are not limited to, the sulfur-containing acids and acid ester groups, thioether groups, mercapto groups and thioketo groups. Moieties containing nitrogen atoms that may replace hydrogen as described in (b) immediately above include, but are not limited to, amino groups, the nitro group, azo groups, ammonium groups, amide groups, azido groups, isocyanate groups, cyano groups and nitrile groups. Moieties containing halogen atoms that may replace hydrogen as described in (b) immediately above include chloro, bromo, fluoro, iodo groups and any of the moieties previously described where a hydrogen or a pendant alkyl group is substituted by a halo group to form a stable substituted moiety.

It is understood that any of the above moieties (b)(i) through (b)(v) can be substituted into each other in either a monovalent substitution or by loss of hydrogen in a polyvalent substitution to form another monovalent moiety that can replace hydrogen in the organic compound or radical.

As used herein, the terms "purified" and "isolated" refer to the removal of contaminants from a sample. For example, perhydrolases are purified by removal of contaminating proteins and other compounds within a solution or preparation that are not perhydrolases. In some embodiments, recombinant perhydrolases are expressed in bacterial or fungal host cells and these recombinant perhydrolases are purified by the removal of other host cell constituents; the percent of recombinant perhydrolase polypeptides is thereby increased in the sample.

As used herein, "protein of interest," refers to a protein (*e.g.*, an enzyme or "enzyme of interest") which is being analyzed, identified and/or modified. Naturally-occurring, as well as recombinant proteins find use in the present invention.

As used herein, "protein" refers to any composition comprised of amino acids and recognized as a protein by those of skill in the art. The terms "protein," "peptide" and polypeptide are used interchangeably herein. Wherein a peptide is a portion of a protein, those skilled in the art understand the use of the term in context.

As used herein, functionally and/or structurally similar proteins are considered to be "related proteins." In some embodiments, these proteins are derived from a different genus and/or species, including differences between classes of organisms (*e.g.*, a bacterial protein and a fungal protein). In some embodiments, these proteins are derived from a different genus and/or species, including differences between classes of organisms (*e.g.*, a bacterial enzyme and a fungal enzyme). In additional embodiments, related proteins are provided from the same species. Indeed, it is not intended that the present invention be limited to related proteins from any particular source(s). In addition, the term "related proteins" encompasses tertiary structural homologs and primary sequence homologs (*e.g.*, the perhydrolase of the present invention). In further embodiments, the term encompasses proteins that are immunologically cross-reactive. In most particularly preferred embodiments, the related proteins of the present invention very high ratios of perhydrolysis to hydrolysis.

As used herein, the term "derivative" refers to a protein which is derived from a protein by addition of one or more amino acids to either or both the C- and N-terminal end(s), substitution of one or more amino acids at one or a number of different sites in the amino acid sequence, and/or deletion of one or more amino acids at either or both ends of the protein or at one or more sites in the amino acid sequence, and/or insertion of one or more amino acids at one or more sites in the amino acid sequence. The preparation of a protein derivative is preferably achieved by modifying a DNA sequence which encodes for the native protein, transformation of that DNA sequence into a suitable host, and expression of the modified DNA sequence to form the derivative protein.

Related (and derivative) proteins comprise "variant proteins." In some preferred embodiments, variant proteins differ from a parent protein and one another by a small number of amino acid residues. The number of differing amino acid residues may be one or more, preferably 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more amino acid residues. In some preferred embodiments, the number of different amino acids between variants is between 1 and 10. In some particularly preferred embodiments, related proteins and particularly variant proteins comprise at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% amino acid sequence identity. Additionally, a related protein or a variant protein as used herein, refers to a protein that differs from another related protein or a parent protein in the number of prominent regions. For example, in some embodiments, variant proteins have 1, 2, 3, 4, 5, or 10 corresponding prominent regions that differ from the parent protein.

Several methods are known in the art that are suitable for generating variants of the perhydrolase enzymes of the present invention, including but not limited to site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches.

In particularly preferred embodiments, homologous proteins are engineered to produce enzymes with the desired activity(ies). In some particularly preferred embodiments, the engineered proteins are included within the SGNH-hydrolase family of proteins. In some most preferred embodiments, the engineered proteins comprise at least one or a combination of the following conserved residues: L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205. In alternative embodiments, these engineered proteins comprise the GDSL-GRTT and/or ARTT motifs. In further embodiments, the enzymes are multimers, including but not limited to dimers, octamers, and tetramers. In yet additional preferred embodiments, the engineered proteins exhibit a perhydrolysis to hydrolysis ratio that is greater than 1.

An amino acid residue of a perhydrolase is equivalent to a residue of *M. smegmatis* perhydrolase if it is either homologous (*i.e.*, having a corresponding position in either the primary and/or tertiary structure) or analogous to a specific residue or portion of that residue in *M. smegmatis* perhydrolase (*i.e.*, having the same or similar functional capacity to combine, react, and/or chemically interact).

In some embodiments, in order to establish homology to primary structure, the amino acid sequence of a perhydrolase is directly compared to the *M. smegmatis* perhydrolase primary sequence and particularly to a set of residues known to be invariant in all perhydrolases for which sequence is known. After aligning the conserved residues, allowing for necessary insertions and deletions in order to maintain alignment (*i.e.*, avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of *M. smegmatis* perhydrolase are defined. In preferred embodiments, alignment of conserved residues conserves 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues are also adequate to define equivalent residues. In preferred embodiments, conservation of the catalytic serine and histidine residues are maintained. Conserved residues are used to define the corresponding equivalent amino acid residues of *M. smegmatis* perhydrolase in other perhydrolases (*e.g.*, perhydrolases from other *Mycobacterium* species, as well as any other organisms).

In some embodiments of the present invention, the DNA sequence encoding *M. smegmatis* perhydrolase is modified. In some embodiments, the following residues are modified: Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196. However, it is not intended that the present invention be limited to sequence that are modified at these positions. Indeed, it is intended that the present invention encompass various modifications and combinations of modifications.

In additional embodiments, equivalent residues are defined by determining homology at the level of tertiary structure for a perhydrolase whose tertiary structure has been determined by x-ray crystallography. In this context, “equivalent residues” are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the carbonyl hydrolase and *M. smegmatis* perhydrolase (N on N, CA on CA, C on C, and O on O) are within 0.13nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the perhydrolase in question to the *M. smegmatis* perhydrolase. As known in the art, the best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available. Equivalent residues which are functionally and/or structurally analogous to a specific residue of *M. smegmatis* perhydrolase are defined as those amino acids of the perhydrolases that preferentially adopt a conformation such that they either alter, modify or modulate the protein structure, to effect changes in substrate binding and/or catalysis in a manner defined and attributed to a specific residue of the *M. smegmatis* perhydrolase. Further, they are those residues of the perhydrolase (in cases where a tertiary structure has been obtained by x-ray crystallography), which occupy an analogous position to the extent that although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two of the side chain atoms of the residue lie with 0.13 nm of the corresponding side chain atoms of *M. smegmatis* perhydrolase. The coordinates of the three dimensional structure of *M. smegmatis* perhydrolase were determined and are set forth herein (*See e.g.*, Example 14) and find use as outlined above to determine equivalent residues on the level of tertiary structure.

In some embodiments, some of the residues identified for substitution, insertion or deletion are conserved residues whereas others are not. The perhydrolase mutants of the present invention include various mutants, including those encoded by nucleic acid that comprises a signal sequence. In some embodiments of perhydrolase mutants that are encoded by such a sequence are secreted by an expression host. In some further embodiments, the nucleic acid sequence comprises a homolog having a secretion signal.

Characterization of wild-type and mutant proteins is accomplished via any means suitable and is preferably based on the assessment of properties of interest. For example, pH and/or temperature, as well as detergent and /or oxidative stability is/are determined in some embodiments of the present invention. Indeed, it is contemplated that enzymes having various degrees of stability in one or more of these characteristics (pH, temperature, proteolytic stability, detergent stability, and/or oxidative stability) will find use. In still other embodiments, perhydrolases with low peracid degradation activity are selected.

As used herein, "expression vector" refers to a DNA construct containing a DNA sequence that is operably linked to a suitable control sequence capable of effecting the expression of the DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself. In the present specification, "plasmid," “expression plasmid,” and "vector" are often used interchangeably as the plasmid is the most commonly used form of vector at present. However, the invention is intended to include such other forms of expression vectors that serve equivalent functions and which are, or become, known in the art.

In some preferred embodiments, the perhydrolase gene is ligated into an appropriate expression plasmid. The cloned perhydrolase gene is then used to transform or transfect a host cell in order to express the perhydrolase gene. This plasmid may replicate in hosts in the sense that it contains the well-known elements necessary for plasmid replication or the plasmid may be designed to integrate into the host chromosome. The necessary elements are provided for efficient gene expression (*e.g.*. a promoter operably linked to the gene of interest). In some embodiments, these necessary elements are supplied as the gene's own homologous promoter if it is recognized, (*i.e.*, transcribed, by the host), a transcription terminator (a polyadenylation region for eukaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the perhydrolase gene. In some embodiments, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media is also included.

The following cassette mutagenesis method may be used to facilitate the construction of the perhydrolase variants of the present invention, although other methods may be used.

First, as described herein, a naturally-occurring gene encoding the perhydrolase is obtained and sequenced in whole or in part. Then, the sequence is scanned for a point at which it is desired to make a mutation (deletion, insertion or substitution) of one or more amino acids in the encoded perhydrolase. The sequences flanking this point are evaluated for the presence of restriction sites for replacing a short segment of the gene with an oligonucleotide pool which when expressed will encode various mutants. Such restriction sites are preferably unique sites within the protein gene so as to facilitate the replacement of the gene segment. However, any convenient restriction site which is not overly redundant in the perhydrolase gene may be used, provided the gene fragments generated by restriction digestion can be reassembled in proper sequence. If restriction sites are not present at locations within a convenient distance from the selected point (from 10 to 15 nucleotides), such sites are generated by substituting nucleotides in the gene in such a fashion that neither the reading frame nor the amino acids encoded are changed in the final construction. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by M13 primer extension in accord with generally known methods. The task of locating suitable flanking regions and evaluating the needed changes to arrive at two convenient restriction site sequences is made routine by the redundancy of the genetic code, a restriction enzyme map of the gene and the large number of different restriction enzymes. Note that if a convenient flanking restriction site is available, the above method need be used only in connection with the flanking region which does not contain a site.

Once the naturally-occurring DNA and/or synthetic DNA is cloned, the restriction sites flanking the positions to be mutated are digested with the cognate restriction enzymes and a plurality of end termini-complementary oligonucleotide cassettes are ligated into the gene. The mutagenesis is simplified by this method because all of the oligonucleotides can be synthesized so as to have the same restriction sites, and no synthetic linkers are necessary to create the restriction sites.

As used herein, "corresponding to," refers to a residue at the enumerated position in a protein or peptide, or a residue that is analogous, homologous, or equivalent to an enumerated residue in a protein or peptide.

As used herein, "corresponding region," generally refers to an analogous position along related proteins or a parent protein.

The terms "nucleic acid molecule encoding," "nucleic acid sequence encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

As used herein, the term "analogous sequence" refers to a sequence within a protein that provides similar function, tertiary structure, and/or conserved residues as the protein of interest (*i.e.,* typically the original protein of interest). For example, in epitope regions that contain an alpha helix or a beta sheet structure, the replacement amino acids in the analogous sequence preferably maintain the same specific structure. The term also refers to nucleotide sequences, as well as amino acid sequences. In some embodiments, analogous sequences are developed such that the replacement amino acids result in a variant enzyme showing a similar or improved function. In some preferred embodiments, the tertiary structure and/or conserved residues of the amino acids in the protein of interest are located at or near the segment or fragment of interest. Thus, where the segment or fragment of interest contains, for example, an alpha-helix or a beta-sheet structure, the replacement amino acids preferably maintain that specific structure.

As used herein, "homologous protein" refers to a protein (*e.g.*, perhydrolase) that has similar action and/or structure, as a protein of interest (*e.g.*, an perhydrolase from another source). It is not intended that homologs be necessarily related evolutionarily. Thus, it is intended that the term encompass the same or similar enzyme(s) (*i.e.*, in terms of structure and function) obtained from different species. In some preferred embodiments, it is desirable to identify a homolog that has a quaternary, tertiary and/or primary structure similar to the protein of interest, as replacement for the segment or fragment in the protein of interest with an analogous segment from the homolog will reduce the disruptiveness of the change. In some embodiments, homologous proteins have induce similar immunological response(s) as a protein of interest.

As used herein, "homologous genes" refers to at least a pair of genes from different species, which genes correspond to each other and which are identical or very similar to each other. The term encompasses genes that are separated by speciation (*i.e.*, the development of new species) (*e.g.*, orthologous genes), as well as genes that have been separated by genetic duplication (*e.g.*, paralogous genes). These genes encode "homologous proteins."

As used herein, "ortholog" and "orthologous genes" refer to genes in different species that have evolved from a common ancestral gene (*i.e.*, a homologous gene) by speciation. Typically, orthologs retain the same function during the course of evolution. Identification of orthologs finds use in the reliable prediction of gene function in newly sequenced genomes.

As used herein, "paralog" and "paralogous genes" refer to genes that are related by duplication within a genome. While orthologs retain the same function through the course of evolution, paralogs evolve new functions, even though some functions are often related to the original one. Examples of paralogous genes include, but are not limited to genes encoding trypsin, chymotrypsin, elastase, and thrombin, which are all serine proteinases and occur together within the same species.

As used herein, "wild-type" and "native" proteins are those found in nature. The terms "wild-type sequence," and "wild-type gene" are used interchangeably herein, to refer to a sequence that is native or naturally occurring in a host cell. In some embodiments, the wild-type sequence refers to a sequence of interest that is the starting point of a protein engineering project. The genes encoding the naturally-occurring protein may be obtained in accord with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protein of interest, preparing genomic libraries from organisms expressing the protein, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.

The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant oligonucleotide" refers to an oligonucleotide created using molecular biological manipulations, including but not limited to, the ligation of two or more oligonucleotide sequences generated by restriction enzyme digestion of a polynucleotide sequence, the synthesis of oligonucleotides (*e.g.*, the synthesis of primers or oligonucleotides) and the like.

The degree of homology between sequences may be determined using any suitable method known in the art (*See e.g.*, Smith and Waterman, Adv. Appl. Math., 2:482 [1981]; Needleman and Wunsch, J. Mol. Biol., 48:443 [1970]; Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 [1988]; programs such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package (Genetics Computer Group, Madison, WI); and Devereux *et al.*, Nucl. Acid Res., 12:387-395 [1984]).

For example, PILEUP is a useful program to determine sequence homology levels. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle, (Feng and Doolittle, J. Mol. Evol., 35:351-360 [1987]). The method is similar to that described by Higgins and Sharp (Higgins and Sharp, CABIOS 5:151-153 [1989]). Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps. Another example of a useful algorithm is the BLAST algorithm, described by Altschul *et al.*, (Altschul *et al.*, J. Mol. Biol., 215:403-410, [1990]; and Karlin *et al.*, Proc. Natl. Acad. Sci. USA 90:5873-5787 [1993]). One particularly useful BLAST program is the WU-BLAST-2 program (*See*, Altschul *et al.*, Meth. Enzymol.,, 266:460-480 [1996]). parameters "W," "T," and "X" determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*See*, Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 [1989]) alignments (B) of 50, expectation (E) of 10, M'5, N'-4, and a comparison of both strands.

As used herein, "percent (%) nucleic acid sequence identity" is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues of the sequence.

As used herein, the term "hybridization" refers to the process by which a strand of nucleic acid joins with a complementary strand through base pairing, as known in the art.

As used herein, the phrase "hybridization conditions" refers to the conditions under which hybridization reactions are conducted. These conditions are typically classified by degree of "stringency" of the conditions under which hybridization is measured. The degree of stringency can be based, for example, on the melting temperature (Tm) of the nucleic acid binding complex or probe. For example, "maximum stringency" typically occurs at about Tm-5°C (5° below the Tm of the probe); "high stringency" at about 5-10° below the Tm; "intermediate stringency" at about 10-20° below the Tm of the probe; and "low stringency" at about 20-25° below the Tm. Alternatively, or in addition, hybridization conditions can be based upon the salt or ionic strength conditions of hybridization and/or one or more stringency washes. For example, 6xSSC = very low stringency; 3xSSC = low to medium stringency; 1xSSC = medium stringency; and 0.5xSSC = high stringency. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe.

For applications requiring high selectivity, it is typically desireable to use relatively stringent conditions to form the hybrids (*e.g.*, relatively low salt and/or high temperature conditions are used).

The phrases "substantially similar and "substantially identical" in the context of at least two nucleic acids or polypeptides typically means that a polynucleotide or polypeptide comprises a sequence that has at least about 40% identity, more preferable at least about 50% identity, yet more preferably at least about 60% identity, preferably at least about 75% identity, more preferably at least about 80% identity, yet more preferably at least about 90%, still more preferably about 95%, most preferably about 97% identity, sometimes as much as about 98% and about 99% sequence identity, compared to the reference (*i.e.*, wild-type) sequence. Sequence identity may be determined using known programs such as BLAST, ALIGN, and CLUSTAL using standard parameters. (*See e.g.*, Altschul, *et al.*, J. Mol. Biol. 215:403-410 [1990]; Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89:10915 [1989]; Karin *et al.*, Proc. Natl. Acad. Sci USA 90:5873 [1993]; and Higgins *et al.*, Gene 73:237 - 244 [1988]). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Also, databases may be searched using FASTA (Pearson *et al.*, Proc. Natl. Acad. Sci. USA 85:2444-2448 [1988]). One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions (*e.g.*, within a range of medium to high stringency).

As used herein, "equivalent residues" refers to proteins that share particular amino acid residues. For example, equivalent resides may be identified by determining homology at the level of tertiary structure for a protein (*e.g.*, perhydrolase) whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the protein having putative equivalent residues and the protein of interest (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins analyzed. The preferred model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available, determined using methods known to those skilled in the art of crystallography and protein characterization/analysis.

As used herein, the terms "hybrid perhydrolases" and "fusion perhydrolases" refer to proteins that are engineered from at least two different or "parental" proteins. In preferred embodiments, these parental proteins are homologs of one another. For example, in some embodiments, a preferred hybrid perhydrolase or fusion protein contains the N-terminus of a protein and the C-terminus of a homolog of the protein. In some preferred embodiment, the two terminal ends are combined to correspond to the full-length active protein.

The term "regulatory element" as used herein refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Additional regulatory elements include splicing signals, polyadenylation signals and termination signals.

As used herein, "host cells" are generally prokaryotic or eukaryotic hosts which are transformed or transfected with vectors constructed using recombinant DNA techniques known in the art. Transformed host cells are capable of either replicating vectors encoding the protein variants or expressing the desired protein variant. In the case of vectors which encode the pre- or prepro-form of the protein variant, such variants, when expressed, are typically secreted from the host cell into the host cell medium.

The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means transformation, transduction or transfection. Means of transformation include protoplast transformation, calcium chloride precipitation, electroporation, naked DNA and the like as known in the art. (*See*, Chang and Cohen, Mol. Gen. Genet., 168:111 - 115 [1979]; Smith *et al.*, Appl. Env. Microbiol., 51:634 [1986]; and the review article by Ferrari *et al.*, *in* Harwood, Bacillus, Plenum Publishing Corporation, pp. 57-72 [1989]).

The term "promoter/enhancer" denotes a segment of DNA which contains sequences capable of providing both promoter and enhancer functions (for example, the long terminal repeats of retroviruses contain both promoter and enhancer functions). The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An endogenous enhancer/promoter is one which is naturally linked with a given gene in the genome. An exogenous (heterologous) enhancer/promoter is one which is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques).

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript. Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York [1989], pp. 16.7-16.8). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

The term "stable transfection" or "stably transfected" refers to the introduction and integration of foreign DNA into the genome of the transfected cell. The term "stable transfectant" refers to a cell which has stably integrated foreign or exogenous DNA into the genomic DNA of the transfected cell.

The terms "selectable marker" or "selectable gene product" as used herein refer to the use of a gene which encodes an enzymatic activity that confers resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed.

As used herein, the terms "amplification" and "gene amplification" refer to a process by which specific DNA sequences are disproportionately replicated such that the amplified gene becomes present in a higher copy number than was initially present in the genome. In some embodiments, selection of cells by growth in the presence of a drug (*e.g.*, an inhibitor of an inhibitable enzyme) results in the amplification of either the endogenous gene encoding the gene product required for growth in the presence of the drug or by amplification of exogenous (*i.e.*, input) sequences encoding this gene product, or both. Selection of cells by growth in the presence of a drug (*e.g.*, an inhibitor of an inhibitable enzyme) may result in the amplification of either the endogenous gene encoding the gene product required for growth in the presence of the drug or by amplification of exogenous (*i.e.*, input) sequences encoding this gene product, or both.

"Amplification" is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (*i.e.*, replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (*i.e.*, synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

As used herein, the term "co-amplification" refers to the introduction into a single cell of an amplifiable marker in conjunction with other gene sequences (*i.e.*, comprising one or more non-selectable genes such as those contained within an expression vector) and the application of appropriate selective pressure such that the cell amplifies both the amplifiable marker and the other, non-selectable gene sequences. The amplifiable marker may be physically linked to the other gene sequences or alternatively two separate pieces of DNA, one containing the amplifiable marker and the other containing the non-selectable marker, may be introduced into the same cell.

As used herein, the terms "amplifiable marker," "amplifiable gene," and "amplification vector" refer to a marker, gene or a vector encoding a gene which permits the amplification of that gene under appropriate growth conditions.

As used herein, the term "amplifiable nucleic acid" refers to nucleic acids which may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" will usually comprise "sample template."

As used herein, the term "sample template" refers to nucleic acid originating from a sample which is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template which may or may not be present in a sample. Background template is most often inadvertent. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

“Template specificity” is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Qβ replicase, MDV-1 RNA is the specific template for the replicase (*See e.g.*, Kacian *et al.*, Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acids are not replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (*See*, Chamberlin *et al.*, Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (*See*, Wu and Wallace, Genomics 4:560 [1989]). Finally, *Taq* and *Pfu* polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the term "target," when used in reference to amplification methods (*e.g.*, the polymerase chain reaction), refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

As used herein, the term "polymerase chain reaction" ("PCR") refers to the methods of U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, which include methods for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified".

As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

The Present Invention

In some most particularly preferred embodiments, the present invention finds use in the enzymatic generation of peracids from ester substrates and hydrogen peroxide. In some preferred embodiments, the substrates are selected from one or more of the following: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. Importantly, the present invention provides means for effective cleaning, bleaching, and disinfecting over broad pH and temperature ranges. In some embodiments, the pH range utilized in this generation is 4-12. In alternative embodiments, the temperature range utilized is between 5° and 90°C. The present invention provides advantages over the presently used systems (*See e.g.*, EP Appln. 87-304933.9) in that bleaching is possible at the optimum pH of peracid oxidation, as well as providing bleaching at neutral pH, acidic pHs, and at low temperatures. While the present invention is described herein most fully in regard to laundry and fabric care, it is not intended that the present invention be limited to these applications. Indeed, the present invention finds use in various settings, particularly those in which bleaching by peracids and/or hydrogen peroxide are desired, including but not limited to laundry, fabric treatment, pulp and paper processing, personal care applications, disinfection and cleaning of hard surfaces. For example, it is contemplated that the compositions of the present invention will find use in bleaching of pulp, including use in methods such as those set forth in U.S. Patent Nos. 6,569,286, 5,785,812, 6,165,318, and 4,400,237, all of which are herein incorporated by reference.

Historically, sodium perborate, and more recently, sodium percarbonate, have been used as bleaching compounds, particularly in European laundry detergents. This compound decomposes rapidly in aqueous solution to yield hydrogen peroxide (H_2O_2), which is the active bleaching species. As sodium perborate is more active at temperatures above 80°C, and less active in the temperature range of 40-60°C (*i.e.*, wash temperatures that have become most commonly preferred as of the 1950s), bleaching activators have been incorporated into laundry detergents that contain sodium perborate. Indeed, most laundry detergents contain bleaching activators. These activators are compounds with O- or N-bounded acetyl groups that are able to react with the strongly nucleophilic hydroperoxy anion to yield peroxyacetic acid. Since the reacting species is hydroperoxy anion, alkaline pHs are essential for the efficient conversion of these activators to peracids. The peroxyacetic acid is decomposed in weakly basic media to form singlet oxygen (*See*, Hofmann *et al.*, J. Prakt. Chem., 334:293-297 [1992]).

Hydrogen peroxide is a particularly effective bleach at high temperatures (*e.g.*, >40°C) and pH (>10), conditions that are typically used in washing fabrics in some settings. However, as indicated above, cold water washing is becoming more commonly used and results in less effective bleaching by H_2O_2 than use of hot water. To overcome this low temperature disadvantage, detergent formulations typically include bleach boosters, such as TAED (N,N,N'N'-tetraacetylethylenediamine), NOBS (nonanoyloxybenzene sulfonate), etc. These boosters combine with H_2O_2 to form peracetic acid, a peracid species that is more effective than H_2O_2 alone. Although it helps the bleaching capability of detergent, the TAED reaction is only approximately 50% efficient, as only two out of the four acetyl groups in TAED are converted to peracids. Additionally, conversion of TAED into peracetic acid by hydrogen peroxide is efficient only at alkaline pHs and high temperatures. Thus, the TAED reaction is not optimized for use in all bleaching applications (*e.g.*, those involving neutral or acidic pHs, and cold water). The present invention provides means to overcome the disadvantages of TAED use. For example, the present invention finds use in cold water applications, as well as those involving neutral or acidic pH levels. Furthermore, the present invention provides means for peracid generation from hydrogen peroxide, with a high perhydrolysis to hydrolysis ratio. The present invention further provides advantages over compositions that contain enzymes such as esterases and lipases) which have very low perhydrolysis to hydrolysis ratios.

In addition to its applications in detergents, the present invention provides methods and compositions for the use of peracids in textile bleaching and in various other applications. In some embodiments, the present invention provides one-step methods for textile processing applications, including but not limited to one-step desizing, scouring and bleaching processes (*See e.g.,* EP WO 03002810, EP 1255888, WO 0164993, and US 20020007516, all of which are hereby incorporated by reference). As described in greater detail herein, in some embodiments, bleaching involves processing textile material before it is dyed and/or after it is incorporated into textile goods. However, it is not intended that the present invention be limited to any particular regimen of use nor any particular textile material.

Furthermore, the peracetic technology of the present invention finds use as an effective bactericide (*See*, Baldry, J. Appl. Bacteriol., 54:417-423 [1983]). Thus, the present invention provides compositions and methods for the sterilization/disinfection of various objects, including but not limited to medical devices, medical equipment, industrial equipment, and fermenters, as well as any additional object that needs to be sterilized or disinfected. As discussed in greater detail below, during the development of the present invention, the enzyme of the present invention was used in a standard cell kill experiment to demonstrate this suitability. In additional embodiments, the present invention provides compositions and methods suitable for use in biofilm control, such as in cooling towers.

Also as described in more detail in the Examples below, the present invention provides many advantages for cleaning and/or sterilization of a wide range of objects, including but not limited to clothing, fabrics, medical devices, etc. In addition, the present invention provides compositions that are effective in cleaning, bleaching, and disinfecting, over a range of wash temperatures and pHs. In additional embodiments, the present invention finds use in degradation of peracids through the perhydrolase peracid degradation activity. In some preferred embodiments, this activity is used in peracid waste clean up applications.

Furthermore, the perhydrolase enzymes of the present invention are active on various acyl donor substrates, as well as being active at low substrate concentrations, and provide means for efficient perhydrolysis due to the high peracid:acid ratio. Indeed, it has been recognized that higher perhydrolysis to hydrolysis ratios are preferred for bleaching applications (*See e.g.*, U.S. Patent No. 5,352,594, 5,108,457, 5,030,240, 3974,082, and 5,296,616, all of which are herein incorporated by reference). In preferred embodiments, the perhydrolase enzymes of the present invention provide perhydrolysis to hydrolysis ratios that are greater than 1. In particularly preferred embodiments, the perhydrolase enzymes provide a perhydrolysis to hydrolysis ratio greater than 1 and are find use in bleaching.

In addition, it has been shown to be active in commonly used detergent formulations (*e.g.*, Ariel Futur, WOB, etc.). Thus, the present invention provides many advantages in various cleaning settings.

As indicated above, key components to peracid production by enzymatic perhydrolysis are enzyme, ester substrate, and hydrogen peroxide. Hydrogen peroxide can be either added directly in batch, or generated continuously "*in situ.*" Current washing powders use batch additions of H_2O_2, in the form of percarbonate or perborate salts that spontaneously decompose to H_2O_2. The perhydrolase enzymes of the present invention find use in the same washing powder batch method as the H_2O_2 source. However, these enzymes also find use with any other suitable source of H_2O_2, including that generated by chemical, electro-chemical, and/or enzymatic means. Examples of chemical sources are the percarbonates and perborates mentioned above, while an example of an electrochemical source is a fuel cell fed oxygen and hydrogen gas, and an enzymatic example includes production of H_2O_2 from the reaction of glucose with glucose oxidase. The following equation provides an example of a coupled system that finds use with the present invention.

$$\text{Glucose} + H_2O \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$+$$

$$H_2O_2 + \text{ester substrate} \xrightarrow{\text{Perhydrolase}} \text{alcohol} + \text{peracid}$$

It is not intended that the present invention be limited to any specific enzyme, as any enzyme that generates H_2O_2 with a suitable substrate finds use in the methods of the present invention. For example, lactate oxidases from *Lactobacillus* species which are known to create H_2O_2 from lactic acid and oxygen find use with the present invention. Indeed, one advantage of the methods of the present invention is that the generation of acid (*e.g.*, gluconic acid in the above example) reduces the pH of a basic solution to the pH range in which the peracid is most effective in bleaching (*i.e.*, at or below the pKa). Other enzymes (*e.g.*, alcohol oxidase, ethylene glycol oxidase, glycerol oxidase, amino acid oxidase, etc.) that can generate hydrogen peroxide also find use with ester substrates in combination with the perhydrolase enzymes of the present invention to generate peracids. In some preferred embodiments, the ester substrates are selected from one or more of the following acids: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. Thus, as described herein, the present invention provides definite advantages over the currently used methods and compositions for detergent formulation and use, as well as various other applications.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention provides methods and compositions comprising at least one perhydrolase enzyme for cleaning and other applications. In some particularly preferred embodiments, the present invention provides methods and compositions for generation of peracids. The present invention finds particular use in applications involving cleaning, bleaching and disinfecting.

Cloning and Characterization of *M. smegmatis* Perhydrolase

The cloning of the *M. smegmatis* perhydrolase (*i.e.*, referred to herein as the "*phd*" gene, which encodes the "Phd" protein; this perhydrolase gene is sometimes herein referred to as the "*act*" gene and the protein is sometimes referred to as the "Act" protein) of the present invention was based on peptide sequence data from the acyltransferase purified from *Mycobacterium parafortuitum* (previously known as *Corynebacterium oxydans*) and published information regarding the 7-aminocephalosporanic acid (7-ACA) arylesterase gene of *Agrobacterium radiobacter* (Sakai *et al.*, J. Ferment. Bioengineer., 85: 138-143 [1998]). Two peptide sequences from purified *M. parafortuitum* acyltransferase were found to be similar to internal N- and C-terminal regions of the *A. radiobacter* 7-ACA-arylesterase (47% and 42% identity respectively).

A set of PCR primers was designed based on the amino acid sequence of these internal peptides (designated "AtintF" and "AtintR"). Another set of primers was developed based on the 5' and 3' ends ("ATNcoI" and "ATBamH1") of the *A. radiobacter* 7-ACA DNA sequence. A single product of the expected size was amplified from *M. parafortuitum* chromosomal DNA using both sets of primers. The full length product, amplified by the ATNcoI/ATBamH1 primer pair, was cloned into pET16b and transformed into BL21 cells (Novagen, Madison, WI). This clone had a sequence identical to that of the *A. radiobacter* 7-ACA gene. As it was determined that purified *M. parafortuitum* perhydrolase was not the 7-ACA acyl esterase, it was concluded that this was not the gene encoding the perhydrolase of the present invention.

Thus, efforts were further focused on *M. smegmatis* for cloning and expression of the perhydrolase of the present invention. To identify the *M. parafortuitum* gene based on enzyme activity screening, a plasmid library of *M. parafortuitum* DNA in *M. smegmatis* was constructed using a plasmid with a promoter to drive expression of cloned genes. Surprisingly, *M. smegmatis* itself was found to be positive for perhydrolase and acyltransferase activity. Thus, in some instances herein, the perhydrolase is referred to as "ACT" (or "Act"). A protein BLAST search of the *M. smegmatis* unfinished genome using the sequence of the *A. radiobacter* 7-ACA identified a 2 kb contig containing an ORF (open reading frame) that encoded a hypothetical protein that was similar but not identical to the 7-ACA protein. Based on this sequence, primers were designed and used to amplify the gene from *M. smegmatis* (ATCC 10143). By adding an *E. coli* ribosome binding site upstream of the start codon, a clone that expressed active enzyme was obtained. The vector used was either pCR2.1TOPO or pBluntIITOPO (Invitrogen, Carlsbad, CA), in *E. coli* Top10 cells. The gene was expressed constitutively from the plasmid-encoded *lac* promoter. This enzyme carried out the same reactions as the originally described *M. parafortuitum* acyltransferase.

During the characterization of the perhydrolase of the present invention, standard protein BLAST searches identified a few proteins (<20) with sequence similarity of 30-80%. This group included the 7-ACA arylesterases from *A. radiobacter* and other organisms, which have 43% identity with *M. smegmatis* perhydrolase. All of the identified homologs with at least 40% similarity have a GDS motif very near the N-terminal end. All of the proteins also contain most of the conserved residues which could place them within the suggested GDSL family of lipolytic enzymes (*See e.g.*, Upton and

Buckley, Trends Biochem. Sci., 20:178 [1995]). However, enzymes mentioned in this paper do not appear on homology searches with the perhydrolase protein. Indeed these proteins have less than 20% similarity with the perhydrolase and its homologs, suggesting that the acyltransferase-related (and perhydrolase of the present invention) enzymes form a subfamily.

The natural function of the enzyme of the present invention and the closely related proteins, apart from the 7-ACA arylesterase, have not been biochemically determined. *M. smegmatis* appears to be the only organism with the acyltransferase/perhydrolase in an operon with a putative penicillin binding protein (PBP). While it is not intended that the present invention be limited to any particular mechanism, this suggests that the enzyme may be involved in cell wall synthesis/structure or modification of molecules taken up from the environment. There are no homologues of the perhydrolase of the present invention that have been identified in *M. tuberculosis* or *M. leprae* to date. However, some organisms were determined to have multiple homologues (*e.g., S. meliloti*).

During the development of the present invention, various mutations were made in the *M. smegmatis* perhydrolase in order to assess its activity. This enzyme contains two cysteine residues, which were hypothesized as potentially forming disulfide bonds, both of which were changed to alanine, in order to determine whether or not the C residues had any effect on the activity of the enzyme. Activity assay results obtained using the transesterification (in aqueous solution) assay described herein indicated that C7A, as well as C77A, and a double mutant (C7A and C77A) were of the same size and specific activity.

Many enzymes have the amino acid serine as part of their active site and are therefore referred to, among other designations, as "serine hydrolases." The active site may consist of a catalytic triad of S (serine), D (aspartic acid) and H (histidine). Examples of such enzymes include, but are not limited to subtilisin (D32-H64-S215), chymotrypsin (H57-D102-S195) and lipases in the alpha/beta hydrolase family (*e.g.*, S126-D176-H206). A typical motif for lipases is the GDSL motif (Upton and Buckley, *supra* [1995]) in which the S is the active site serine. Since the perhydrolase of the present invention was determined to have a GDSL (amino acids 9-12) motif, the S11 was mutated to an A, in order to confirm the involvement of this S in the active site. As indicated in the Examples, the activity assay results indicated that S11A had only 1% of the activity of the wild-type enzyme. Deletion of the C-terminal 25 amino acids also resulted in abrogation of the activity, suggesting that these amino acids either contained a residue involved directly in the active site, and/or that the structure of the protein was affected such that the active site was no longer able to catalyze the reactions. In addition, the predicted active site residues, D192 and H195 were mutated to A. Neither mutant had activity, confirming that the active site residues of the perhydrolase of the present invention consist of S11, D192 and H195. However, it is not intended that the present invention be limited to any particular mechanism, nor is the present invention limited to mutation(s) at any particular active site residues.

Cloning of *M. parafortuitum* Perhydrolase

There were some differences between the N-terminal peptide sequence obtained from the *M. parafortuitum* enzyme and the N-terminal sequence of *M. smegmatis* perhydrolase. However, there was a sequence in the C-terminal region of the *M. smegmatis* perhydrolase identical to the C-terminal peptide sequence of the *M. parafortuitum* enzyme. Two primers were designed to amplify a partial sequence of the *M. parafortuitum* perhydrolase gene; the sequence of the reverse primer was identical to the sequence of the corresponding region in *M. smegmatis* perhydrolase gene, and the sequence of the forward primer was based on *M. smegmatis* codon usage. The forward primer, MP5: 5'-ATGGGTACCCGACGAATTCTGTCCTTCGGTGATTCCCTGACCT-3' (SEQ ID NO:11) and the reverse primer MPC-intR 5'-

GATTCCGTCGACGCCGTCGGTGCTGATCACCGAACCCGCGTCGAAGAACGG-3' (SEQ ID NO:12). The partial gene was amplified from the chromosome of *M. parafortuitum* and cloned into pCR2.1TOPO (Invitrogen, Carlsbad, CA). Sequence analysis showed that the enzyme is very similar, but not identical to the *M. smegmatis* perhydrolase (77% identity). Based on the molecular weights of the monomers of the perhydrolases determined by SDS-PAGE (MP AT: 26 kDa, MSAT: 24 kDa, MP cloned AT: ~18 kDa), the clone from primers made to the internal fragment was determined to be missing approximately 70 amino acids (~8 kDa). The remaining sequence at the 5'-end of the *M. parafortuitum* gene can be obtained by any of several methods suitable and familiar to those skilled in the art of molecular biology, including, but not limited to, inverse PCR, probing of plasmid/cosmid libraries of *M. parafortuitum* chromosomal DNA, sequencing of the gene directly from chromosomal DNA (*e.g.*, as performed by Fidelity Systems, Bethesda Maryland).

Expression of the *M. smegmatis* Perhydrolase

The perhydrolase is an intracellular protein in its native host. Production of the perhydrolase in non-native hosts may also be done intracellularly. However, in some embodiments, a signal sequence is added to the perhydrolase, which facilitates expression of the perhydrolase by secretion into the periplasm (*i.e.*, in Gram-negative organisms, such as *E. coli*), or into the extracellular space (*i.e.*, in Gram-positive organisms, such as *Bacillus* and *Actinomycetes*), or eukaryotic hosts (*e.g.*, *Trichoderma*, *Aspergillus*, *Saccharomyces*, and *Pichia*). Of course, these are just a few examples of possible prokaryotic and eukaryotic hosts. It is not intended that the present invention be limited to these specific hosts, as various other organisms find use as expression hosts in the present invention.

A variety of commercially available expression systems, including but not limited to pBAD, plac, T7, find use in the expression of the perhydrolase in Gram-negative hosts (*e.g.*, *E. coli*). In some embodiments, the same types of promoters find use in another Gram-negative host, *Pantoea citrea*.

To test expression in *E. coli* two strategies were used: 1) adding an RBS (ribosome binding site) to the 5' end of the *phd* gene and cloning the gene into pCRBLUNTIITOPO (Invitrogen), thus allowing expression directly from the pLac promoter available in that vector; and 2) cloning the *phd* gene under control of the T7 promoter in the plasmid pET16b (Novagen). In the latter system, expression of the gene is inducible by addition of IPTG to the growing culture and use of a specific host cell (*e.g.*, BL21(λDE3)pLysS (Novagen)) that contains the λDE3 lysogen encoding the T7 RNA polymerase. The first strategy produces a plasmid capable of allowing expression of the perhydrolase protein in other Gram-negative hosts (*e.g.*, *P. citrea*).

To express protein in *E. coli* or *P. citrea* using the first strategy, cultures were grown from single, purified colonies at 37°C overnight in L broth plus the appropriate antibiotic (example, kanamycin 50 μg/ml). Expression of the protein was determined by the pNB assay (*See*, Example 1) after lysis of the cells.

Expression of the perhydrolase using the T7 expression system requires induction of the culture with the addition of IPTG (*e.g.*, 100 mmole IPTG added at an OD_{550} of 0.4). Overnight cultures, inoculated from a single colony, are used to inoculate the expression culture of the desired volume (25 mls to several liters) at an OD_{550} of 0.1. The expression culture was then grown at the desired temperature (*e.g.*, 25°C, 30°C, 37°C) until an OD_{550} of 0.4 was reached, after which IPTG was added. Expression was allowed to continue for 3 hours to overnight. Protein expression was monitored by pNB activity assay as described in Example 1. Usually, expression from the T7 system gives a high titer of protein, sufficient for further analysis such as crystallography.

Bacillus species are well-known as suitable hosts for expression of extracellular proteins (*e.g.*, proteases). Intracellular expression of proteins is less well known. Expression of the perhydrolase protein intracellularly in *Bacillus subtilis* can be done using a variety of promoters, including, but not limited to pVeg, pSPAC, pAprE, or pAmyE in the absence of a signal sequence on the 5' end of the gene. In some embodiments, expression is achieved from a replicating plasmid (high or low copy number), while in alternative embodiments, expression is achieved by integrating the desired construct into the chromosome. Integration can be done at any locus, including but not limited to the *aprE*, *amyE*, or *pps* locus. In some embodiments, the perhydrolase is expressed from one or more copies of the integrated construct. In alternative embodiments, multiple integrated copies are obtained by the integration of a construct capable of amplification (*e.g.*, linked to an antibiotic cassette and flanked by direct repeat sequences), or by ligation of multiple copies and subsequent integration into the chromosome. In some embodiments, expression of the perhydrolase with either the replicating plasmid or the integrated construct is monitored using the pNB activity assay (described herein) in an appropriate culture.

As with *Bacillus*, in some embodiments, expression of the perhydrolase in the Gram-positive host *Streptomyces* is done using a replicating plasmid, while in other embodiments, expression of the perhydrolase is accomplished via integration of the vector into the *Streptomyces* chromosome. Any promoter capable of being recognized in *Streptomyces* finds use in driving transcription of the perhydrolase gene (*e.g.*, glucose isomerase promoter, A4 promoter). Replicating plasmids, either shuttle vectors or *Streptomyces* only, also find use in the present invention for expression (*e.g.*, pSECGT).

Structure of *M. smegmatis* Perhydrolase

The crystal structure of the *M. smegmatis* perhydrolase was determined to 2.2 Angstroms. The structure confirmed findings with gel filtration sizing columns, that indicated this enzyme is an octamer. The structure of the monomer places the enzyme in the class known as SGNH-hydrolases (*See e.g.*, Molgaard *et al.*, Structure 8: 373-383 [2000]). The active site residues were identified as S11-D192-H195, based on homology, confirming the identification of the catalytic triad based on loss of activity in the S11A, D192A, and H195A mutations described above. Figure 3 provides schematics showing the structure of the *M. smegmatis* perhydrolase, as well as other serine hydrolases. As indicated, this enzyme has a different structure than the enzymes shown here (chymotrypsin, subtilisin, and α/β hydrolase). Indeed, the structural analysis of the perhydrolases of the present invention indicates that this group of enzymes has a different form and active site than do these other enzymes. A schematic diagram of the structure of the monomer is illustrated in Figure 4. The structures of four other enzymes in the SGNH-hydrolase family have been solved, namely *Aspergillus aculeatus* rhamnogalucturonan acetylesterase (RGAE), *Bos taurus* platelet activating factor (PAF-AH(1b)a), *Streptomyces scabies* esterase (SsEst) and the thioesterase/Protease I/Phospholipase L_1 (TAP or Tes) from *E. coli*. Very little sequence or functional homology is present in these enzymes. Basically, the sequence identity is reserved for the residues involved in the active site and those defining the family. While the overall folding of the enzymes is similar (*See e.g.*, Molgaard *et al.*, *supra* [2000], for overlaying of structures), there are structural differences. For example, there is a loop covering the active site in SsEst, compared to RGAE and TAP which have active sites that are surface-exposed. The *M. smegmatis* perhydrolase has an active site that is somewhat buried. The binding residues of the *M. smegmatis* perhydrolase were identified as Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196. These sites were derived from direct observation and by modeling studies to model substrate binding to the enzyme, using methods known in the art.

As indicated above, the *M. smegmatis* perhydrolase was found to be an octamer in the crystalline state. However, it is contemplated to be either a hexamer or octamer in solution. The octamer is seen to be a tetramer of dimers, two molecules are much more closely and extensively interacting and these are termed the "act transferase" dimers. Several of the conserved sites are found along this dimer interface. For example, residues Trp 14, Arg 27, Arg 56, His 81 and Pro 83, were found to be conserved in natural isolates that have perhydrolase activity and are contemplated to be critical in forming the interface. In addition one other residue, Glu 51, which is conserved in all but one of the natural isolates (and in that case it is a homologous enzyme) was identified.

One additional feature of interest in that in the natural isolates showing perhydrolase activity, all share an insertion of residues 69-81. This region forms a loop that is at the dimer interface. Without this loop, it is believed that much of the dimer interface would be lost and it is likely that dimers and subsequent aggregation would not occur. Thus, there is a correlation of the insertion with the structural aggregation particularly dimer formations and the appearance of perhydrolase activity. However, it is not intended that the present invention be limited to any particular mechanisms.

Key residues were found to be associated with desired activity in selected homologs. Indeed, there are several conserved residues that are contemplated to have importance for acyltransferase activity. These include Leu 6, Trp 14, Arg 27, Trp 34, Asp 62, Leu74, Leu 78 His 81, Pro83, Met 90, Lys 97, and Leu 114.

In additional analyses, the association of the perhydrolase with carbamate was investigated. The native octamer was determined in space group P4 with unit cell dimensions:

a= 98.184 b= 98.184 and c= 230.119 α=90.00 β=90.00 γ=90.00, this crystal diffracted to about 2.0 Å. The carbamate-inhibited crystal grew in the space group P1 with unit cell dimensions a=67.754, b=80.096, and c=85.974 α=104.10°, β=112.10°, and γ=97.40° and these crystals diffract to a resolution exceeding 1.0 Å.

The carbamate was bound in a manner to exploit the interactions between the keto oxygen of the carbamate and residues forming the oxyanion hole, the amide N atoms of Ser 11 and Ala 55 and Asn 94 ND2. The hydrophobic side chain extends along the hydrophobic surface of the binding site out into the surface opening between pairs of dimers in the octamer structure. The carbamate moiety direction highlights the pivotal role of the S54V mutation. The hydrophobic moiety passes adjacent to the side chain of ser 54. Mutating the serine side to valine increased the hydrophobicity, and also served as a gatekeeper to prevent hydrophilic nucleophiles (*e.g.*, water) for competing with desired deacylating nucleophiles. The residues surrounding the carbamate moiety on the same and neighboring molecules forming the extended entry are expected to influence the selection of the optimal de-acylating nucleophile. The structure showed that each monomer was inhibited with carbamate covalently attached. Thus, all octamer active sites were found to be active and functional. The side chain of carbamate resembles the leaving groups of the substrates tested. Thus, the carbamate moiety indicates the access direction for substrate.

M. smegmatis Perhydrolase is an SGNH-Hydrolase

The perhydrolase of the present invention has certain components that indicate it is in the SGNH-hydrolase family of enzymes. This family is defined by having the four conserved amino acids SGN and H in four blocks, similar to the blocks that describe the lipolytic family of enzymes (*See*, Upton and Buckley, *supra*). In the case of the *M. smegmatis* perhydrolase, these correspond to S11, G52, N94 and H195 which correspond to Blocks I II, III and V according to Upton and Buckley (Upton and Buckley, *supra*) and Molgaard *et al.* (Molgaard *et al.*, *supra*). These amino acids are also conserved within the closest sequence homologs of the perhydrolase.

As indicated herein, the sequences were aligned using the Alignment program in Vector NTi (Informax, Invitrogen) In the following alignment providing a comparison of homolog sequences, the double underline indicates the residues involved in the active site. AR: *Agrobacterium rhizogenes* Q9KWA6; RR: *Rhizobium rhizogenes* NF006; SM: *Sinorhizobium meliloti* RSM02162; MS: *Mycobacterium smegmatis* Act; MP:

Mycobacterium parafortuitum Phd partial sequence; PD: *Prosthecobacter dejongeii* RVM04532. The amino acids within the blocks defining the SGNH-hydrolase family are indicated in bold letters.

```
                             Block I                                     Block II
                               GDS                                          G
AR(1) ----------MAESRSILCFGDSLTWGWIPVPESSP TLRYPFEQRWTGAMAAALGDGYSIIEEGLSARTTSVED--PN
RR(1) ----------MAESRSILCFGDSLTWGWIPVPESSP TLRYPFEQRWTGAMAAALGDGYSIIEEGLSARTTSVED-PN
RM(1)MTINSHSWRTLMVEKRSVLCFGDSLTWGWIPVKESSP TLRYPYEQRWTGAMAARLGDGYHIIEEGLSARTTSLDD-PN
SM(1)-----------MVEKRSVLCFGDSLTWGWIPVKESSP TLRYPYEQRWTGAMAARLGDGYHIIEEGLSARTTSLDD-PN
MS(1)-------------MAKRILCFGDSLTWGWVPVEDGAP TERFAPDVRWTGVLAQQLGADFEVIEEGLSARTTNIDD-PT
MP   -------------GTRRILSFGDSLTWGWIPVEEGVP TERFPRDVRWTGVLADLLGDRYEVIEEGLSARTTTAED- PA
PD(1)--------------MKTILCFGDSNTWGYDPASMTAPFPRRHGPEVRWTGVLAKALGAGFRVIEEGQNGRTTVHED--PL

                                        Block III
                                          GxND
AR(67)  DPRLNGSAYLPMALASHLPLDLVIILLGTNDTKSYFRRTPYEIANGMGKLAGQVLTSAGGIGTPYPAPKLLIVSPPPLAP
RR(67)  DPRLNGSAYLPMALASHLPLDLVIILLGTNDTKSYFRRTPYEIANGMGKLAGQVLTSAGGIGTPYPAPKLLIVSPPPLAP
RM(78)  DARLNGSTYLPMALASHLPLDLVIIMLGTNDTKSYFHRTPYEIANGMGKLVGQVLTCAGGVGTPYPAPKVLVVAPPPLAP
SM(67)  DARLNGSTYLPMALASHLPLDLVIIMLGTNDTKSYFHRTPYEIANGMGKLVGQVLTCAGGVGTPYPAPKVLVVAPPPLAP
MS(65)  DPRLNGASYLPSCLATHLPLDLVIIMLGTNDTKAYFRRTPLDIALGMSVLVTQVLTSAGGVGTTYPAPKVLVVSPPPLAP
MP(65)  DPRLNGSQYLPSCLASHLPLDLVILMLGTNDTKANFGRTPFDIATGMGVLATQVLTSAGGVGTSYPAPQVLIVAPPPLGE
PD(65)  NICRKGKDYLPACLESHKPLDLVILMLGTNDLKSTFNVPPGEIAAGAGVLGRMILAGDAGP-ENRPPQLLLMCPPKVRDL

                                                             Block V
                                                              DGIHF
AR(147) MPDPWFEGMFGGGYEKSLELAKQYKALANPLKVDFLDAGEFVKTDGCDGIHFSAETNITLGHAIAAKVEAIFSQEAKNAA(SEQ ID NO:14)
RR(147) MPDPWFEGMFGGGYEKSLELAKQYKALANPLKVDFLDAGEFVKTDGCDGIHFSAETNITLGHAIAAKVEAIFSQEAKNAA(SEQ ID NO:15)
RM(158) MPDPWFEGMFGGGYEKSKELSGLYKALADFMKVEFFAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF--------(SEQ ID NO:16)
SM(147) MPDPWFEGMFGGGYEKSKELSGLYKALADFMKVEFFAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF--------(SEQ ID NO:17)
MS(145) MPHPWFQLIFEGGEQKTTELARVYSALASFMKVPFFDAGSVISTDGVDGIHFTEANNRDLGVALAEQVRSLL--------(SEQ ID NO:18)
MP 145) LPHPWFDLVFSGGREKTAELARVYSALASFMKVPFFDAGSVISTDGVDGI-----------------------------(SEQ ID NO:19)
PD(144) SAMPDLDAKIPHGAARSAEFPRHYKAQAVALKCEYFNSQEIVETSPVDGIHLEASEHLKLGEALAEKVKVLLG-------(SEQ ID NO:20)
```

The primers used to identify homologs for each of the Blocks indicated above are provided below:

Block I (forward 5´-3)

1e: acggtcctgtgctttggngaytcnyt (SEQ ID NO:21)
1f: acggtcctgtgctttggngayagyyt (SEQ ID NO:22)

1g: gcggtcctgttctwnggngaytcnyt (SEQ ID NO:23)
1h: gcggtcctgttctwnggngayagyyt (SEQ ID NO:24)
1i: gctcgaaccgtcctctgttttggngaytcnyt (SEQ ID NO:25)
1j: gctcgaaccgtcctctgttttggngayagyyt (SEQ ID NO:26)
1k: gctcgaaccgtcctctgtttnggngaytc (SEQ ID NO:27)
1l: gctcgaaccgtcctctgttttggngaytcnytn (SEQ ID NO:28)
1m: gctcgaaccgtcctctgttttggngaytcnytg (SEQ ID NO:29)
1A: gccaagcgaattctgtgtttcggngaytcnyt (SEQ ID NO:30)
1B: gccaagcgaattctgtgtttcggngayagyyt (SEQ ID NO:31)

Block III (reverse 5′-3)

3c: attccgcgcttcagrtcrttnvtncc (SEQ ID NO:32)
3d: attccgcgcttcagrtcrttnwgncc (SEQ ID NO:33)
3e: attccgcgcttcagrtcrttnscncc (SEQ ID NO:34)
3f: attccgcgcttcagrtcrttnrancc (SEQ ID NO:35)
3k: attccgcgcttcagrtcrttnrtncc (SEQ ID NO:36)
3l: attccgcgcttcagrtcrttnytncc (SEQ ID NO:37)
3m: attccgcgcttcagrtcrttnsgncc (SEQ ID NO:38)
3n: attccgcgcttcagrtcrttnwcncc (SEQ ID NO:39)
3o: attccgcgcttcagrtcrttnyancc (SEQ ID NO:40)
3p: attccgcgcttgrsrtcrttnrtncc (SEQ ID NO:41)
3q: attccgcgcttgrsrtcrttnytncc (SEQ ID NO:42)
3r: attccgcgcttgrsrtcrttnsgncc (SEQ ID NO:43)
3s: attccgcgcttgrsrtcrttnwcnnn (SEQ ID NO:44)
3t: attccgcgcttgrsrtcrttnyancc (SEQ ID NO:45)
3A: gcgccggaagtaggccttggtrtcrttnvtncc (SEQ ID NO:46)
3B: gcgccggaagtaggccttggtrtcrttnwgncc (SEQ ID NO:47)
3C: gcgccggaagtaggccttggtrtcrttnscncc (SEQ ID NO:48)
3D: gcgccggaagtaggccttggtrtcrttnrancc (SEQ ID NO:49)

Block III (forward 5′-3)

3g: cggaattatcatgctgggnabnaayga (SEQ ID NO:50)
3h: cggaattatcatgctgggncwnaayga (SEQ ID NO:51)
3i: cggaattatcatgctgggngsnaayga (SEQ ID NO:52)
3j: cggaattatcatgctgggntynaayga (SEQ ID NO:53)
3u: ccggaattatcatgctnggnabnaayga (SEQ ID NO:54)
3v: ccggaattatcatgctnggncwnaayga (SEQ ID NO:55)
3w: ccggaattatcatgctnggngsnaayga (SEQ ID NO:56)
3x: ccggaattatcatgctnggntynaayga (SEQ ID NO:57)

Block V (reverse 5′-3)

5c: acccttagcgtttggrtgnrtnccrtc (SEQ ID NO:58)
5d: atccttagcgtttggrtgnavnccrtc (SEQ ID NO:59)
5e: aatcttagccgtgrrrtgnrtnccrtc (SEQ ID NO:60)
5f: aatcttagccgtgrrrtgnrcnccrtc (SEQ ID NO:61)
5g: aatcttagccgtgrrrtgntrnccrtc (SEQ ID NO:62)
5h: ccgctggtcctcatctggrtgnrtnccrtc (SEQ ID NO:63)
5i: ccgctggtcctcatctggrtgnrcnccrtc (SEQ ID NO:64)
5j: ccgctggtcctcatctggrtgntrnccrtc (SEQ ID NO:65)
5k: ccgctggtcctcatcraartgnrtncc (SEQ ID NO:66)
5A: cgattgttcgcctcgtgtgaartgnrtnccrtc (SEQ ID NO:67)
5B: cgattgttcgcctcgtgtgaartgnrcnccrtc (SEQ ID NO:68)
5C: cgattgttcgcctcgtgtgaartgntrnccrtc (SEQ ID NO:69)

As described in greater detail herein, the sequence and structure results are supported by the activity data that indicate the perhydrolase enzymes of the present invention differ from lipolytic enzymes known in the art.

Identification of Homologs

As well known in the art, proteins with a desired activity may be identified in several ways, including but not limited to: 1) searching available databases for proteins with sequence homology (30-100%); 2) screening environmental isolates for the desired activity; and 3) examining type strains from ATCC of the genus identified to have activities (*e.g., Mycobacterium* and *Corynebacterium*, as described herein in particular embodiments).

By doing a standard protein-protein BLAST search, several homologs were identified from fully or partially sequenced genomes. From the known gene sequence, several homologs were amplified by PCR from the chromosome of the parent organism and cloned into a pET expression vector, essentially as described for the cloning of *phd* from *M. smegmatis* into pET16b. Homologues identified by this BLAST search included: *Agrobacterium rhizogenes* Q9KWA6, *A. rhizogenes* Q9KWB1 *A. tumefaciens* Q8UFG4, *A. tumefaciens* Q8UAC0 (now AgrL, identical to 7-ACA arylesterase), *A. tumefaciens* Q9ZI09, *A. tumefaciens* (radiobacter)ACA, *Prosthecobacter. dejongeii* RVM04532 , *Rhizobium. loti* Q98MY5 , *R. meliloti* Q92XZ1, *R. meliloti* Q9EV56, *R. rhizogenes* NF006, *R. rhizogenes* NF00602875, *R. solanacerarum* Q8XQI0, *Sinorhizobium meliloti* RSM02162, *S. meliloti* RSM05666, *Mesorhizobium loti* RMLO00301, *A. rhizogenes* Q9KWA6, and *A. rhizogenes* Q9KWB1.

Based on these results, a homology tree of proteins with sequence homology (20-80%) to *M. smegmatis* perhydrolase was generated. As shown in Figure 2, an enzyme in the family of lipolytic enzymes described by Upton and Buckley (*supra*) is that of *V. mimicus*. This phylogenetic tree was generated using the alignment program in Vector NTi (Informax, Invitrogen). The green arrow indicates *M. smegmatis* perhydrolase, the red arrow indicates *A. radiobacter* 7-ACA arylesterase, the blue arrow indicates *E. coli* TAP, and the black arrow indicates *A. aculeatus* RGAE.

As further indicated in Figure 2, the perhydrolase is not closely related to this enzyme. The perhydrolase and its closest relatives, *Prosthecobacter dejongeii* RVM04532, *R. rhizogenes* NF006, *A. rhizogenes* Q9KWA6, *R. meliloti* Q92XZ1, *S. meliloti* RSM02162, *A. rhizogenes* Q9KWB1 and *R. rhizogenes* NF00602875 come off their own branch (*i.e.*, a branch that is different from the 7-ACA arylesterase-like proteins and the RGAE/TAP-like proteins). However, it is contemplated that some additional, more distantly related homologs will find use in the present invention due to perhydrolase activity or will serve as a suitable backbone for modification to the desired perhydrolase activity.

In addition to the sequence and homology analysis, environmental isolates were grown on a rich medium (N-MISO: g/l: glucose 10 g, yeast extract 10 g, KNO_3 1.5,

KH_2PO_4 3.4 g, $NaH_2PO_4.H_20$ 3.4 g, Salt Solution C 10 ml [Salt Solution C: g/l: $MgSO_47H_2O$ 25, $FeSO_47H_2O$ 2.8, $MnSO_4H_2O$ 1.7, NaCl 0.6, $NaMoSO_4.2H_2O$, $ZnSO_4.7H_2O$ 0.06, in 0.1N HCl]), assayed and those positive for the transesterification reaction were purified as described in the Examples. This is one of the screening methods that can be used to identify perhydrolase These data show that the present invention finds use in identification of additional enzymes with the desired perhydrolase activity.

Additional Investigations of Homologues

In addition to the above analyses, an enzyme library of novel "GDSL-type" esterases which are homologous to the prototype *M. smegmatis* perhydrolase was created. In order to identify new "GDSL"-type esterases, a sequence homology based screening procedure was established and used to screen libraries set up from complex metagenomic DNA (at BRAIN).

An enzyme library comprising 19 "GDSL"-type esterases (*See,* below) was developed. The sequences in this library were:

S248_M2bB11 (DNA)
ATGTTCGCGCTTTGCACGGCCGCGTCAGCGGCCCCCGATCGCACCGTCGTCTT
TTTTGGGGACAGCCTGACCGCGGGGTACGGCCTCGATGACCCGCAGACCCAG
TCCTACCCGGCCAGGATCCAGGAGAAGGTCGACGCCGCGGGCCTGCGCTGGA
AGGTCGTGAATGCCGGCCTCTCGGGCGAGACGAGCGCCGGCGGCCTGCGGCG
GGTCGACTGGGTGCTCGGCCAGCACATCGACGCCTTTGTCCTGGCGCTTGGCG
CCAACGATGGCCTGCGGGGGATCGACCCCCAGGTCACGAGGGCCAATCTCCA
GGAGATCATCAACCGGGTCCGCTCCCGGTGGCCCCGCGCGGCGATCGTCATC
GCCGGGATGAAAATGCCCCAGAGCATGGGACAGGACTACGCCGCGAATTTTG
ACCGGATCTTCCCCGGTCTCGCCGCGAGGAATTCGGCCACGCTCATCCCCTTT
CTATTAGAAGGGGTCGCCGCCCATCCTAGCCTCAACCAAGGCGACGGCATCC
ACCCGACGGCCGCCGGGGACGCACTCGTTGCAGGGACCGTGTGGACGTACCT
GCTTCCGATCCTGCGGTCAGCACACTAA (SEQ ID NO:70)

S248_M2bB11 (Amino Acid)
MFALCTAASAAPDRTVVFFGDSLTAGYGLDDPQTQSYPARIQEKVDAAGLRWK
VVNAGLSGETSAGGLRRVDWVLGQHIDAFVLALGANDGLRGIDPQVTRANLQEII
NRVRSRWPRAAIVIAGMKMPQSMGQDYAANFDRIFPGLAARNSATLIPFLLEGV
AAHPSLNQGDGIHPTAAGDALVAGTVWTYLLPILRSAH (SEQ ID NO:71)

S248_M40cD4 (DNA)
ATGCGCTTTGCTAAGCTCACTGCCGTCATCTTTGCCCTGATAGTCTTGCACAG
CCCCCTTGCCGCCGCCGCGCCGCCCACCGTGATGGTGTTTGGCGACAGTCTGA
CCGCCGGGTTGGGATTGCCGGCCGATGCTGCATTTCCGGCGCAGCTCCAGGC
AAAGCTGCACGATATGGGTATCCTGCAGAAATCGCCGCGCGCGCCACCTCGG
GGCAAACGACGGCCGGCGGGTTGGCGAGCCTTGCGGATGCGCTGGCCGCAA
AGCCGGATTTGGTGATCCTCGAACTCGGCGCCAATGACATGCTGCGCGCGGT
CGATCCGGCCAGCGTGCGCGCCAATCTCGATGCAATGATGACGAAAATCCAG
GCGAGCGGCGCTAAACTGCTGCTGACCGGAATGCAGGCGGCGCCCAATTGGG
GCGAGGACTATAAGCACGATTTCGACCGCCTTTATCCCGAGCTTGCGAAGGC
GCACGGGGTGACGCTTTATCCATTCTTTCTTGATGGGGTGGCGCTGGACCCGG
CGCTGAACCAGGCGGATGGAATGCACCCGAACGCCAAGGGGGTCGCCGTGA
TCGTCGACCGTATCGCGCCCGTCGTCGCCAAGATGCTGAGAGGCCAGTCATA
A (SEQ ID NO:72)

S248_M40cD4 (Amino Acid)
MRFAKLTAVIFALIVLHSPLAAAAPPTVMVFGDSLTAGLGLPADAAFPAQLQAKL
HDMGIPAEIAARATSGQTTAGGLASLADALAAKPDLVILELGANDMLRAVDPAS
VRANLDAMMTKIQASGAKLLLTGMQAAPNWGEDYKHDFDRLYPELAKAHGVT
LYPFFLDGVALDPALNQADGMHPNAKGVAVIVDRIAPVVAKMLRGQS (SEQ ID NO:73)

S248_M44aA5 (DNA)
ATGATCGCATGGCTTACCGGATGCGGCAGCGCAAAGACGCAACCGCAGCCCG
CAAGTTCCATCCCGCCATCCAGTATTCCAGCAACCGCAAAACCTGCGACAAC
GGATATCAGACCGATCATCGTTGCTTTCGGCGACAGCCTGACTGCAGGATAC
GGCGTCAGTAGTGAACAAAGCTATCCGGCCAATCTTCAACGCGATCTGGATG
CGCGTGGATATCATGCCCACGTCATCAACGAAGGCATCAGCGGCAACACATC
GAAAGACGGCGTTCTCAGGGCCCAGGCGATTGCGGCACTCCATCCGGCTGTC
GTCATCGTTGCCTTCGGCGGCAACGACGGTCTGCGTGGCCTCCCCATCGGAG
ACACGGAAATGAATCTGGCAACGATCATCTCAACCATGCAGCATGCCCATGC
CAAGGTAATTTTAGGCGGAATTACTTTGCCTCCCAACTATGGCAGCGAATAC

ATCGCCAAATTCAATGCGATCTATAAAAAGCAGGCAGCCGCGTATCATGTGC
CCCTGCTGCCCTTCATGCTGAAGGGGGTGTATGGCGTGCCCGGTTCCATGCAG
AGCGACGGCATCCATCCGACCGCCAAGGGCTGCCAGCAAGTGGCCAGAAACT
TCCTGCCCTTGTTATTGCCGCTCCTGCACAAATCAGGGAAGAAATCCATGGAG
TCGAAAGCATTGTCTCGACGTCATTAA (SEQ ID NO:74)

S248_M44aA5 (Amino Acid)
MIAWLTGCGSAKTQPQPASSIPPSSIPATAKPATTDIRPIIVAFGDSLTAGYGVSSEQ
SYPANLQRDLDARGYHAHVINEGISGNTSKDGVLRAQAIAALHPAVVIVAFGGN
DGLRGLPIGDTEMNLATIISTMQHAHAKVILGGITLPPNYGSEYIAKFNAIYKKQA
AAYHVPLLPFMLKGVYGVPGSMQSDGIHPTAKGCQQVARNFLPLLLPLLHKSGK
KSMESKALSRRH (SEQ ID NO:75)

S261_M2aA12 (DNA)
ATGAAAAACATCCTTGCATTTGGCGACAGTCTGACCTGGGGTTTTGTGGCCGG
ACAGGATGCGCGCCATCCGTTTGAAACCCGCTGGCCAAACGCATTGGCGGCC
GGCCTTGGGGGCAAAGCCCGCGTAATTGAAGAGGGTCAGAACGGCCGCACT
ACGGTGTTCGACGATGCCGCCACCTTCGAATCTCGAAATGGCTCGGTGGCATT
GCCGCTGCTACTGATCAGCCACCAGCCGTTGGACCTGGTAATCATCATGCTCG
GCACCAATGACATCAAGTTTGCCGCCCGCTGCCGCGCCTTTGATGCTTCAATG
GGCATGGAACGGCTGATCCAGATCGTCAGAAGTGCCAACTACATGAAGGGCT
ACAAGATACCTGAAATCCTCATCATATCGCCGCCCAGCCTCGTGCCGACGCA
GGATGAATGGTTCAACGACCTCTGGGGCCATGCCATCGCCGAGTCAAAACTC
TTCGCCAAGCACTACAAGCGCGTGGCCGAAGAACTGAAAGTGCATTTCTTTG
ATGCAGGCACGGTGGCCGTCGCCGACAAGACCGACGGCGGACATCTCGATGC
TGTGAATACTAAAGCCATTGGCGTCGCATTGGTGCCGGTGGTGAAATCAATA
CTCGCTCTCTAA (SEQ ID NO:76)

S261_M2aA12 (Amino Acid)
MKNILAFGDSLTWGFVAGQDARHPFETRWPNALAAGLGGKARVIEEGQNGRTT
VFDDAATFESRNGSVALPLLLISHQPLDLVIIMLGTNDIKFAARCRAFDASMGMER
LIQIVRSANYMKGYKIPEILIISPPSLVPTQDEWFNDLWGHAIAESKLFAKHYKRVA
EELKVHFFDAGTVAVADKTDGGHLDAVNTKAIGVALVPVVKSILAL (SEQ ID NO:77)

S279_M70aE8 (DNA)
ATGCCGAAAATAGCCAAACTCGCGCCGTCGGATGTGATCGTAGCTTTCGGCG
ACAGTCTGACGTTCGGCACCGGCGCAACGGAAGCGGAGAGTTATCCCATCGT
GCTCGCACAATTGATCGGTCGCACCGTGGTGCGCGCGGGTGTGCCGGGTGAG
GTAACCGAAGGCGGGCTTGCGCGCCTGACCGACGTTATCGAAGAACACAAGC
CGAAGCTGATTATTGTTTGCCTGGGCGGCAACGACATGCTGCGCAAGGTCCA
GGAAGACCAGACCCGCGCCAATTTGCGCGCCATTATTAAAACCATCAAGGCG
CAAGGCATCGCCGTGGTACTGGTCGGTGTGCCGAAGCCCGCGCTGGTGACCA
GTGCGCCGCCGTTCTACGAGGAGATCGCCAAAGAGTTCGGTATCCCTTACGA
AGGCAAGATTGTTACCGACGTGTTGTACCAACGCGATCAGAAATCCGATTCC
ATACATCCCAATGCCAAAGGCTATCGGCGCATGGCCGAAGCGATAGCCACGC
TGCTGAAAAAATCCGGAGCCATTTAA (SEQ ID NO:78)

S279:M70aE8 (Amino Acid)
MPKIAKLAPSDVIVAFGDSLTFGTGATEAESYPIVLAQLIGRTVVRAGVPGEVTEG
GLARLTDVIEEHKPKLIIVCLGGNDMLRKVQEDQTRANLRAIIKTIKAQGIAVVLV
GVPKPALVTSAPPFYEEIAKEFGIPYEGKIVTDVLYQRDQKSDSIHPNAKGYRRMA
EAIATLLKKSGAI (SEQ ID NO:79)

S279_M75bA2 (DNA)
ATGGAACGGACCGGCCGCGCTGGCGATCGGTGTCGGCGTGGGGCTGGCGAGC
CTGAGCCCGGTCGCGCTGGCGACGCCGCCGCGGGGCACCGTGCCGGTGTTCA
CCCGATCGGGGACAGCCTGACGGACGAGTATTTTGAGCCGTTCTTCCAGTGG
GGGTTCTGCGGGAAGTCGTGGGCCGAGATTTTGGTGGAGACGGGGCGGGCGA
GCATGGGCCCGACGGCGCAGCAGGCGGGGATCAGCGAGCCGGAGGGATGGT
CGGATCCGCGGAACACGGGGTATCAGCACAACTGGGCGCGGTACTCGTGGAG
CTCCTCAGACGCGCTGACCGAGGAGTCGCCGGGGGCGACGCTGAGCGTGCTG
CTTGGGGCGGAGTACGCGGTGGTGTTCATTGGGACCAACGACTTCAATCCGT
CGTGGCCGGCGTATCAGAGCGTGTATCTGAGCCAGTGGAGCGACGAGCAGAT
CGACACGTACGTGAACGGGGTGGTGCAGAACATCGCGCAGATGGTGGACTCG
CTGAAGTCGGTCGGGGCGAAGGTGGTGCTTGCGCCGCCGGTGGATTTTCAGT
TCGCGGGGTTCCTGCGGAACTCATGCCCGGATCCGATGCTGCGCGAGCAGGC
GGGTATTCTGACACGGAAGTGCCACGACCGGGTGCGGTCGATGGCGCGGCAG
AAGCACGTGGTGTTCGTGGACATGTGGCGGCTGAACCGCGATTTGTTCGGCA
ACGGGTTCGCGATCAGCTACGGCCTTCGGAACACGGTGCGCGTGGGGGACTC
GGAGATCGGGCTGCAACTGGCCGGGCTGACGGGATCGGCGGGGCTGGTTCCG
GACGGGATCCATCCGCAGCGGGTGGTGCAGGGGATCTGGGCGAATGCGTTCA

TCGTGGGTCTGAACGCGCATGGGGCGAACATCGCGCCCATCGGCGAGGCGGA
GATGTGCGCGATGGGGGGGGTCGTGTACGGGGGAACGGACACGCTGGCGAA
CTTCCTGCCGCCGGTCGCGGGCTACGTGGAGGACTTCCGCAACGCGGGGGAC
TTCGTGTGCACGGCGGACTTCAACCATGACCTTGGCGTGACGCCGACGGACA
TCTTCGCGTTCATCAACGCGTGGTTCATGAATGATCCCTCGGCGCGGATGAGC
AACCCGGAGCACACGCAGATCGAGGACATCTTCGTGTTTCTGAATCTGTGGC
TGGTGGGGTGCTAA (SEQ ID NO:80)

S279_M75bA2 (Amino Acid)
MERTGRAGDRCRRGAGEPEPGRAGDAAAGHRAGVHPIGDSLTDEYFEPFFQWG
FCGKSWAEILVETGRASMGPTAQQAGISEPEGWSDPRNTGYQHNWARYSWSSS
DALTEESPGATLSVLLGAEYAVVFIGTNDFNPSWPAYQSVYLSQWSDEQIDTYVN
GVVQNIAQMVDSLKSVGAKVVLAPPVDFQFAGFLRNSCPDPMLREQAGILTRKC
HDRVRSMARQKHVVFVDMWRLNRDLFGNGFAISYGLRNTVRVGDSEIGLQLAG
LTGSAGLVPDGIHPQRVVQGIWANAFIVGLNAHGANIAPIGEAEMCAMGGVVYG
GTDTLANFLPPVAGYVEDFRNAGDFVCTADFNHDLGVTPTDIFAFINAWFMNDP
SARMSNPEHTQIEDIFVFLNLWLVGC (SEQ ID NO:81)

M091_M4aE11 (DNA)
ATGAAGACCATTCTCGCCTATGGCGACAGCCTGACCTATGGGGCCAACCCGA
TCCCGGGCGGGCCGCGGCATGCCTATGAGGATCGCTGGCCCACGGCGCTGGA
GCAGGGGCTGGGCGGCAAGGCGCGGGTGATTGCCGAGGGGCTGGGTGGTCG
CACCACGGTGCATGACGACTGGTTTGCGAATGCGGACAGGAACGGTGCGCGG
GTGCTGCCGACGCTGCTCGAGAGCCATTCGCCGCTCGACCTGATCGTCATCAT
GCTCGGCACCAACGACATCAAGCCGCATCACGGGCGGACGGCCGGCGAGGC
CGGGCGGGGCATGGCGCGGCTGGTGCAGATCATCCGCGGGCACTATGCCGGC
CGCATGCAGGACGAGCCGCAGATCATCCTCGTGTCGCCGCCGCCGATCATCC
TCGGCGACTGGGCGGACATGATGGACCATTTCGGCCCGCACGAAGCGATCGC
CACCTCGGTGGATTTCGCTCGCGAGTACAAGAAGCGGGCCGACGAGCAGAAG
GTGCATTTCTTCGACGCCGGCACGGTGGCGACGACCAGCAAGGCCGATGGCA
TCCACCTCGACCCGGCCAATACGCGCGCCATCGGGGCAGGGCTGGTGCCGCT
GGTGAAGCAGGTGCTCGGCCTGTAA (SEQ ID NO:82)

M091_M4aE11 (Amino Acid)
MKTILAYGDSLTYGANPIPGGPRHAYEDRWPTALEQGLGGKARVIAEGLGGRTT
VHDDWFANADRNGARVLPTLLESHSPLDLIVIMLGTNDIKPHHGRTAGEAGRGM

ARLVQIIRGHYAGRMQDEPQIILVSPPPIILGDWADMMDHFGPHEAIATSVDFARE
YKKRADEQKVHFFDAGTVATTSKADGIHLDPANTRAIGAGLVPLVKQVLGL
(SEQ ID NO:83)

Est105 (DNA)
ATGCGCACGCTTCACCGAAGCCTGCTCGCAAGCGCGGCCGCGCTTTTTCTAGC
GGCATCCGGCAACGCAACGGCGCAGTTCTCGAACGTCTATTTCTTCGGCGAC
AGCCTGACCGACGCGGGTTCCTTCAAGCCTGTGCTGCCTCCTGGTACAGGATT
ATTCACGACGAATCCCGGCCCGGTATGGCCGCAGGTATTCGGGGCGAACTAC
GGCGTCGCGGTGACGCCCGCAAACCAGGGTGGGACCGATTATGCGCAGGGTG
GCGCGCGCGTGACGAGCCTGCCTGGCGTTCCGACGTCGCAGCCGACCGGCAG
CGCGGTACCGATCGCTACGCAGATTTCGCAGTTCCTCGGCTCGGGTCCGGCG
GATCCGAACGCATTCTATTCGGTGTGGGGCGGCGCGAACGACATCTTTTTCCA
GCTGGGGTTGGCGCAGGCGGGCATGGCGACGCCGGCGCAGGTCCAGTCGGCC
GTCGGCTTGGCCGCGGTCCAGCTGGCGCAGGCAACTGCGGCGCTCAACGCCA
GCGGCGCGCGATTCATCACGGTTATCAACGTGCCGGACATCGGTAAAACGCC
GTTCGGCGTCGGCTCCGGTCAAGGAGCGCAGATCACCGCTCTGTCGTCTTTCT
TCAACAGCACGCTGTTCGGCGCGCTCGACGCCACGGGCATCCAGACGATGCG
CGTGAACGGGTTCGCGGTGCTGAACGAGGTGGTCGCGGACCCGGCGGCTTAT
GGCTTCGCGAATGCATCAACGCCAGCGTGCGGGGCCACGCCATCGCTCGTCT
GCACGTCGGCGAACTTCGTCACGCCCTTGGCCGCGCAGACCTTCCTCTTCGCA
GACGGCGTTCACCCCACCACGGCCGGGCACGCCCTCATCGCCCAAGCGGTCC
AGGCGATGATCACCGGTCCCCAACAGATGGCGGCGTTGGGCGACGCCCCGCT
CGCCGTCGAGCAGGCCAACTTCCGCGCGCTCGACAACCGCATGTGGTCGAGC
CTCAATGCGCCGCGCAGCCCGGGCAAGCTCCAGGGTTGGGCGGCCTACGACT
ACAGCCACACGGACCTGCAGGCGGGACCGACCAATGGCAGCGGACACATGA
ACACCGTTGCGGTCGGGGTCGACATGAAAGTCTCCGATCATATGCTCGCCGG
CGCGATGTTCGGCTATACCAACACCAAGGGCGACTTCGGCGGCCCCGGCGGC
GGATACACACTGAAGCAGCCTGTGGGCACTGCCTATGCGGGTTACGGCGTGG
GCCCTTGGTATGTCGGCGCGACGCTCGGCACAGGTGGCCTCGACTACTCGGA
CGTCACGCGCGCCATCCCGCTTGGCTTGGCGGTTCGCACCGAGAGCGCCGAG
GCCCGAGGCTACGAGTTCACGGGCCGGATCCTCGGCGGCTACTGGTTCACGA
TGCGCGACCTGATGCACGGGCCGTACGCGCGTCTCGCGTGGACGAAGGCCGT
CGTCAAGCGGTTTTCCGAGGAGAGCACCGACAGCACGGCGTTGAACTACGAC
AGGCAGGAGCGCAAGCAACTGCTGTGGAGCCTCGGATGGCAACTCGCCGGC
AACGTCGGCAGCATCCGTCCCTACGCGCGGGCGACCTGGGAGATCGACTCCA
AGGATCAGGACCGCAGCGTTGGCGCATCGTCGGTCACGCTGGGCGGCTTTTA
CAGTGTTCCGGTCGCGAAGCCGGACAATAGCTATGCGCTCTTCAGCCTCGGC

GCGAGTACCGAGCTCGGGAGCGTCACCGGGTTTGTCGCGGGCTCGGCCACCG
CAGGCCGGGCGGATGCCAACTATTGGGCGGTCACGGTCGGCCTGCGGATGCC
GTTGTAG (SEQ ID NO:84)

Est105 (Amino Acid)
MRTLHRSLLASAAALFLAASGNATAQFSNVYFFGDSLTDAGSFKPVLPPGTGLFT
TNPGPVWPQVFGANYGVAVTPANQGGTDYAQGGARVTSLPGVPTSQPTGSAVPI
ATQISQFLGSGPADPNAFYSVWGGANDIFFQLGLAQAGMATPAQVQSAVGLAAV
QLAQATAALNASGARFITVINVPDIGKTPFGVGSGQGAQITALSSFFNSTLFGALD
ATGIQTMRVNGFAVLNEVVADPAAYGFANASTPACGATPSLVCTSANFVTPLAA
QTFLFADGVHPTTAGHALIAQAVQAMITGPQQMAALGDAPLAVEQANFRALDN
RMWSSLNAPRSPGKLQGWAAYDYSHTDLQAGPTNGSGHMNTVAVGVDMKVS
DHMLAGAMFGYTNTKGDFGGPGGGYTLKQPVGTAYAGYGVGPWYVGATLGT
GGLDYSDVTRAIPLGLAVRTESAEARGYEFTGRILGGYWFTMRDLMHGPYARLA
WTKAVVKRFSEESTDSTALNYDRQERKQLLWSLGWQLAGNVGSIRPYARATWE
IDSKDQDRSVGASSVTLGGFYSVPVAKPDNSYALFSLGASTELGSVTGFVAGSAT
AGRADANYWAVTVGLRMPL (SEQ ID NO:85)

Est114 (DNA)
ATGGGGCGATCGAGAGTTCTGAAGGCTGTTTTCCTGGTGGCGTGCCTTGTGGG
TCGGCTCGCGGCGCATGCCGAGGCGTCGCCCATCGTGGTCTACGGCGATAGC
CTCTCTGACAACGGCAATCTGTTTGCGCTCACCGGCGGTGTCGCGCCGCCCTC
GCCGCCGTACTTCAACGGACGGTTTTCTAATGGCCCGGTGGCCGTGGAGTATC
TCGCGGCCGCGCTGGGATCTCCGCTGATCGATTTCGCGGTCGGCGGGGCGAC
GACCGGCCTCGGCGTCAACGGCGATCCCGGTGGTTCGCCGACGAGTCTCGGC
GCGGCGGGATTGCCGGGGCTTCAGACGACATTCGCCGCCACGCAAGGCACGC
TGGGTCCGTACGTTGGTGGTCTCTTCGTGGTGTGGGCGGGTCCGAACGACTTC
TTGTCGCCCTCGCCGCTTGACACGAACGCTTTTCAGATTGCGAACCGGGCCGT
GTCCAACATCCTCGGCGTGGTGGCATCACTTCAGGCACTCGGCGTCGAGCGC
ATCCTCGTCCCCGGCATGCCCGATCTCGGTCTGACGCCCGCTCTTCAGCCCAT
CGCAGGCGCAGCCACCGCGTTCACCGATTTGTTCAACTCGATGCTGCGCGCG
GGCTTGCCGAACGACGTGCTGTACCTGGACACGGCGACAATCTTCCGATCGA
TCGTGGCAGACCCTGGGGCCTACGGCTTGACCAACGTGACCACGCCGTGCCT
GATTGGTGCGACCGTCTGCGCGAATCCGGATCAGTACCTGTTCTGGGATGGT
ATTCATCCTACGACGGCGGGGCACGCGATCTTGGGCAATGCCCTCGTCGCCC
AGGCAGTCCCCGAGCCCGCGACCATGGTGCTCGTGCTGACGGGTCTGTCCAT
GCACGTGATTGCGCGCCGGCGGCGGGCGTAA (SEQ ID NO:86)

Est114 (Amino Acid)
MGRSRVLKAVFLVACLVGRLAAHAEASPIVVYGDSLSDNGNLFALTGGVAPPSP
PYFNGRFSNGPVAVEYLAAALGSPLIDFAVGGATTGLGVNGDPGGSPTSLGAAGL
PGLQTTFAATQGTLGPYVGGLFVVWAGPNDFLSPSPLDTNAFQIANRAVSNILGV
VASLQALGVERILVPGMPDLGLTPALQPIAGAATAFTDLFNSMLRAGLPNDVLYL
DTATIFRSIVADPGAYGLTNVTTPCLIGATVCANPDQYLFWDGIHPTTAGHAILGN
ALVAQAVPEPATMVLVLTGLSMHVIARRRRA (SEQ ID NO:87)

Sinorhizobium meliloti SmeI (SMa1993) (DNA)
ATGACAATCAACAGCCATTCATGGAGGACGTTAATGGTGGAAAAGCGCTCAG
TACTGTGCTTTGGGGATTCGCTGACATGGGGCTGGATTCCGGTGAAGGGATC
CTCACCGACCTTGCGCTATCCCTATGAACAACGGTGGACCGGCGCAATGGCC
GCGAGGCTTGGCGACGGTTACCACATCATCGAAGAGGGGCTGAGCGCCCGCA
CCACCAGCCTCGACGACCCCAACGACGCGCGGCTCAACGGCAGCACCTACCT
GCCCATGGCACTCGCCAGCCACCTCCCACTCGACCTCGTCATCATCATGCTGG
GCACGAACGACACGAAATCCTATTTCCACCGCACGCCTTACGAGATCGCCAA
CGGCATGGGCAAGCTAGTCGGCCAGGTGCTGACCTGCGCCGGTGGCGTCGGC
ACGCCATATCCCGCGCCGAAGGTGCTTGTCGTCGCTCCGCCGCCGCTCGCGCC
GATGCCCGACCCGTGGTTCGAAGGCATGTTCGGCGGCGGCTACGAGAAGTCG
AAGGAACTCTCCGGCCTCTACAAGGCGCTTGCCGATTTCATGAAGGTCGAGT
TTTTCGCCGCCGGTGATTGCATTTCCACCGATGGGATCGACGGCATTCACCTC
TCGGCGGAAACCAACATCAGACTCGGGCACGCGATCGCGGACAAAGTTGCG
GCGTTGTTC (SEQ ID NO:88)

Sinorhizobium meliloti SmeI (SMa1993) (Amino Acid)
MTINSHSWRTLMVEKRSVLCFGDSLTWGWIPVKGSSPTLRYPYEQRWTGAMAA
RLGDGYHIIEEGLSARTTSLDDPNDARLNGSTYLPMALASHLPLDLVIIMLGTNDT
KSYFHRTPYEIANGMGKLVGQVLTCAGGVGTPYPAPKVLVVAPPPLAPMPDPWF
EGMFGGGYEKSKELSGLYKALADFMKVEFFAAGDCISTDGIDGIHLSAETNIRLG
HAIADKVAALF (SEQ ID NO:89)

Sinorhizobium meliloti SmeII (Q92XZ1) (DNA)
ATGGAGGAGACAGTGGCACGGACCGTTCTATGCTTCGGAGATTCCAACACTC
ACGGCCAGGTACCTGGCCGCGGACCGCTTGATCGCTACCGACGCGAACAGCG
CTGGGGCGGTGTTCTGCAAGGCCTGCTCGGCCCGAACTGGCAGGTTATCGAA
GAAGGCCTGAGCGGACGCACGACCGTGCATGACGATCCGATCGAAGGTTCGC
TCAAGAACGGCCGGACCTATCTGCGCCCCTGTCTGCAGAGCCATGCACCACT

CGACCTTATCATCATTATGCTCGGCACCAATGACCTGAAGCGGCGCTTCAACA
TGCCACCGTCCGAGGTCGCAATGGGCATCGGCTGTCTCGTGCACGATATCCG
AGAACTCTCGCCCGGCCGGACCGGCAACGATCCCGAAATCATGATCGTCGCC
CCGCCGCCGATGCTGGAAGATCTCAAGGAATGGGAGTCGATTTTCTCAGGCG
CACAGGAAAAATCTCGCAAGCTGGCGCTGGAGTTCGAGATAATGGCGGATTC
TCTGGAGGCGCATTTCTTCGACGCCGGTACGGTCTGCCAGTGTTCGCCGGCCG
ATGGCTTCCACATCGACGAGGATGCCCACCGCCTGCTCGGCGAGGCTCTCGC
CCAGGAAGTGCTGGCGATCGGGTGGCCCGATGCGTAA (SEQ ID NO:90)

Sinorhizobium meliloti SmeII (Q92XZ1) (Amino Acid)
MEETVARTVLCFGDSNTHGQVPGRGPLDRYRREQRWGGVLQGLLGPNWQVIEE
GLSGRTTVHDDPIEGSLKNGRTYLRPCLQSHAPLDLIIIMLGTNDLKRRFNMPPSE
VAMGIGCLVHDIRELSPGRTGNDPEIMIVAPPPMLEDLKEWESIFSGAQEKSRKLA
LEFEIMADSLEAHFFDAGTVCQCSPADGFHIDEDAHRLLGEALAQEVLAIGWPDA
(SEQ ID NO:91)

Sinorhizobium meliloti SmeIII (Q9EV56) (DNA)
ATGAAGACAGTCCTTTGCTACGGTGACAGTCTGACCTGGGGATACGATGCAA
CCGGTTCCGGCCGGCATGCGCTGGAGGACCGTTGGCCGAGCGTGCTGCAGAA
GGCGCTCGGTTCGGACGCGCATGTCATCGCCGAAGGGCTGAACGGGCGGACG
ACCGCCTATGACGACCATCTCGCCGATTGCGACCGGAACGGCGCGCGTGTCC
TCCCGACGGTCCTGCACACCCACGCGCCACTCGATCTCATCGTGTTCATGCTC
GGCTCGAACGACATGAAGCCGATCATTCACGGCACCGCTTTCGGCGCGGTGA
AGGGCATCGAGCGCCTCGTCAATCTGGTGCGCAGGCACGACTGGCCGACGGA
AACGGAGGAGGGGCCCGAGATTCTCATCGTCTCGCCGCCGCCGCTCTGCGAG
ACGGCCAACAGCGCCTTTGCCGCCATGTTCGCGGGCGGGGTCGAGCAATCCG
CAATGCTGGCGCCGCTTTATCGCGATCTCGCCGACGAGCTCGACTGCGGCTTC
TTCGACGGCGGATCGGTGGCCAGGACGACGCCGATCGACGGTGTCCACCTCG
ACGCGGAGAACACCCGGGCGGTCGGCAGAGGGTTGGAGCCTGTCGTGCGGA
TGATGCTCGGGCTTTAA (SEQ ID NO:92)

Sinorhizobium meliloti SmeIII (Q9EV56) (Amino Acid)
MKTVLCYGDSLTWGYDATGSGRHALEDRWPSVLQKALGSDAHVIAEGLNGRTT
AYDDHLADCDRNGARVLPTVLHTHAPLDLIVFMLGSNDMKPIIHGTAFGAVKGIE
RLVNLVRRHDWPTETEEGPEILIVSPPPLCETANSAFAAMFAGGVEQSAMLAPLY
RDLADELDCGFFDGGSVARTTPIDGVHLDAENTRAVGRGLEPVVRMMLGL
(SEQ ID NO:93)

Agrobacterium tumefaciens Atu III (AAD02335) (DNA)
ATGGTGAAGTCGGTCCTCTGCTTTGGCGATTCCCTCACCTGGGGATCAAATGC
GGAAACGGGTGGCCGGCACAGCCATGACGATCTTTGGCCGAGCGTCTTGCAG
AAGGCGCTCGGTCCTGACGTGCATGTGATTCACGAAGGTCTGGGTGGTCGCA
CCACCGCCTATGACGACAACACCGCCGATTGCGACCGCAACGGCGCGCGGGT
TCTTCCGACGTTGTTGCACAGCCATGCGCCGCTGGATCTGGTGATTGTCATGC
TCGGGACCAACGACCTGAAGCCGTCAATCCATGGATCGGCGATCGTTGCCAT
GAAGGGTGTCGAAAGGCTGGTGAAGCTCACGCGCAACCACATCTGGCAGGTG
CCGGACTGGGAGGCGCCTGACGTGCTGATCGTCGCACCGCCGCAGCTGTGTG
AAACGGCCAATCCGTTCATGGGCGCGATCTTTCGTGATGCGATCGATGAATC
GGCGATGCTGGCGTCCGTTTACCGGGACCTTGCCGACGAGCTTGATTGCGGCT
TTTTCGATGCGGGTTCCGTCGCCCGAACGACGCCGGTGGATGGCGTTCATCTC
GATGCTGAAAATACGCGGGCCATCGGGCGGGGGCTGGAGCCCGTCGTTCGCA
TGATGCTCGGACTTTAA (SEQ ID NO:94)

Agrobacterium tumefaciens Atu III (AAD02335) (Amino Acid)
MVKSVLCFGDSLTWGSNAETGGRHSHDDLWPSVLQKALGPDVHVIHEGLGGRT
TAYDDNTADCDRNGARVLPTLLHSHAPLDLVIVMLGTNDLKPSIHGSAIVAMKG
VERLVKLTRNHIWQVPDWEAPDVLIVAPPQLCETANPFMGAIFRDAIDESAMLAS
VYRDLADELDCGFFDAGSVARTTPVDGVHLDAENTRAIGRGLEPVVRMMLGL
(SEQ ID NO:95)

Mesorhizobium loti Mlo I (Q98MY5) (DNA)
ATGAAGACGGTGCTTTGCTACGGCGACTCGCTGACCTGGGGCTACAATGCCG
AAGGCGGCCGCCATGCGCTGGAAGACCGCTGGCCGAGCGTGCTGCAAGCAG
CGTTAGGCGCCGGCGTGCAAGTGATTGCCGATGGCCTCAACGGCCGCACCAC
GGCCTTCGACGATCATCTGGCCGGTGCTGATCGCAACGGCGCCAGGCTGCTG
CCGACGGTCCTGACGACGCACGCGCCGATCGACCTGATCATCTTCATGCTCG
GCGCCAACGACATGAAGCCTTGGATCCACGGCAATCCGGTCGCAGCCAAGCA
AGGCATCCAGCGGTTGATCGACATCGTGCGTGGTCACGACTACCCGTTCGAC
TGGCCGGCGCCGCAGATCCTGATCGTCGCGCCGCCTGTAGTCAGCCGCACCG
AAAATGCCGACTTCAAGGAAATGTTCGCCGGTGGCGATGACGCCTCGAAGTT
TTTGGCACCGCAATATGCCGCGCTCGCCGACGAAGCCGGCTGTGGCTTCTTCG
ACGCCGGCAGCGTGGCCCAAACCACACCGCTCGATGGCGTTCACCTCGATGC
CGAAAACACGCGAGAAATCGGCAAGGCGCTGACGCCGATCGTGCGCGTCAT
GCTGGAATTGTAA (SEQ ID NO:96)

Mesorhizobium loti Mlo I (Q98MY5) (Amino Acid)
MKTVLCYGDSLTWGYNAEGGRHALEDRWPSVLQAALGAGVQVIADGLNGRTT
AFDDHLAGADRNGARLLPTVLTTHAPIDLIIFMLGANDMKPWIHGNPVAAKQGIQ
RLIDIVRGHDYPFDWPAPQILIVAPPVVSRTENADFKEMFAGGDDASKFLAPQYA
ALADEAGCGFFDAGSVAQTTPLDGVHLDAENTREIGKALTPIVRVMLEL (SEQ ID NO:97)

Moraxella bovis Mbo (AAK53448) (DNA)
ATGAAAAAATCCGCCTTTGCCAAATACTCAGCACTTGCCCTAATGGTTGGGAT
GTGCCTGCACACCGCTTACGCCAAGGAGTTTAGCCAAGTCATCATTTTTGGGG
ACAGCTTGTCCGATACAGGTCGCCTAAAAGATATGGTCGCCCGAAAAGATGG
CACCCTTGGCAACACCTTACAGCCATCTTTTACCACCAACCCCGACCCTGTAT
GGTCAAGCTTATTTGCCCAAAGTTATGGCAAAACCGCCAGTCCCAACACGCC
TGACAATCCCACTGGCACTAACTATGCCGTGGGCGGAGCTCGCTCTGGCTCG
GAGGTCAATTGGAATGGTTTTGTGAATGTACCCTCCACCAAAACGCAAATCA
CCGACCATTTGACCGCCACAGGTGGCAAAGCCGACCCTAATACCCTGTATGC
CATTTGGATTGGCTCTAATGACTTAATTTCAGCTTCTCAAGCCACCACAACAG
CCGAAGCCCAAAACGCCATTAAAGGTGCGGTAACTCGCACCGTGATAGACAT
CGAAACACTCAATCAAGCAGGGGCGACAACCATTTTGGTGCCAAATGTGCCT
GATTTGAGCCTCACGCCCCGAGCCATCTATGGCGAAAGCCTCATGGCAGGCG
TGCAAGACAAAGCCAAACTCGCCTCAAGTCTGTATAATAGCGGTCTGTTTGA
AGCATTAAATCAATCCACCGCCAACATCATCCCTGCCAACACCTTTGCCCTAC
TCCAAGAAGCGACCACAAATAAAGAAGCCTTTGGTTTTAAAAACACGCAAGG
CGTGGCGTGTCAAATGCCCGCTCGTACCACAGGGGCGGATGATGTGGCTTCT
ACTTCCTTGGCATGTACCAAAGCCAATCTTATAGAAAACGGGGCAAATGACA
CCTACGCCTTTGCCGATGACATTCACCCATCGGGACGCACGCACCGCATTTTG
GCACAGTATTACCGTTCTATCATGGACGCCCCTACTCACATGGGTAAACTCTC
AGGCGAGCTTGTCAAAACAGGTTCAGCCCACGACCGTCATGTTTACCGTCAG
CTTGACAGGCTTAGTGGCTCACAGCACAGCATTTGGGCAAACGTCTATGCCA
GCGACCGTACCGACCCCACCACCCAAATCGGCTTGGACGTGGCAGGTTCATC
AAGCCATACAGGGGCGTATCTGAGCCACCAAAACCAAGATTATGTGCTGGAT
GACACCCTATCATCAGATGTCAAAACCATTGGCATGGGGCTGTATCATCGCC
ATGACATCGGCAATGTCCGTCTAAAAGGCGTGGCAGGTATCGACCGACTTAG
CGTGGATACGCACCGCCATATCGACTGGGAGGGGACAAGCCGTTCGCACACC
GCAGATACCACCGCCAGACGTTTTCATGCAGGGCTACAAGCCAGCTATGGCA
TAGACATGGGCAAAGCCACCGTGCGTCCGCTTATCGGCGTACATGCCCAAAA
AGTCAAAGTAAATGACATGACCGAGAGCGAATCAACTTTATCCACCGCCATG

CGTTTTGGCGAGCAAGAACAAAAGTCCCTACAAGGCGAGATTGGCGTCGATG
TGGCTTATCCGATTAGCCCTGCTTTGACTCTGACGGGCGGTATCGCTCACGCT
CATGAGTTTAACGATGATGAACGCACCATTAATGCCACTTTAACCTCCATTCG
TGAATACACGAAGGGCTTTAATACAAGCGTTAGCACCGACAAATCTCACGCC
ACCACCGCTCATCTGGGCGTACAAGGGCAACTTGGCAAGGCAAATATTCATG
CAGGCGTTCACGCCACCCACCAAGACAGCGATACAGACGTGGGTGGTTCGCT
TGGGGTTCGCTTGATGTTTTAA (SEQ ID NO:98)

Moraxella bovis Mbo (AAK53448) (Amino Acid)
MKKSAFAKYSALALMVGMCLHTAYAKEFSQVIIFGDSLSDTGRLKDMVARKDG
TLGNTLQPSFTTNPDPVWSSLFAQSYGKTASPNTPDNPTGTNYAVGGARSGSEVN
WNGFVNVPSTKTQITDHLTATGGKADPNTLYAIWIGSNDLISASQATTTAEAQNA
IKGAVTRTVIDIETLNQAGATTILVPNVPDLSLTPRAIYGESLMAGVQDKAKLASS
LYNSGLFEALNQSTANIIPANTFALLQEATTNKEAFGFKNTQGVACQMPARTTGA
DDVASTSLACTKANLIENGANDTYAFADDIHPSGRTHRILAQYYRSIMDAPTHMG
KLSGELVKTGSAHDRHVYRQLDRLSGSQHSIWANVYASDRTDPTTQIGLDVAGS
SSHTGAYLSHQNQDYVLDDTLSSDVKTIGMGLYHRHDIGNVRLKGVAGIDRLSV
DTHRHIDWEGTSRSHTADTTARRFHAGLQASYGIDMGKATVRPLIGVHAQKVKV
NDMTESESTLSTAMRFGEQEQKSLQGEIGVDVAYPISPALTLTGGIAHAHEFNDD
ERTINATLTSIREYTKGFNTSVSTDKSHATTAHLGVQGQLGKANIHAGVHATHQD
SDTDVGGSLGVRLMF (SEQ ID NO:99)

Chromobacterium violaceum Cvi (Q7NRP5) (DNA)
ATGCGCTCTATCGTCTGCAAAATGCTGTTCCCTTTGTTGCTGCTGTGGCAGCT
GCCCGCCCTGGCCGCCACCGTGCTGGTGTTCGGCGACAGCCTGTCCGCCGGC
TACGGCCTGGCCCCGGGCCAGGGATGGGCGGCGCTGCTGGCGCGCGACCTCT
CGCCCCGGCACAAGGTGGTCAACGCCAGCGTGTCCGGCGAAACCAGCGCCGG
CGGCCTGTCCAGGCTGCCCGACGCGCTCGCCCGCCACCAGCCCGACGTGCTG
GTGCTGGAACTCGGCGCCAACGATGGCCTGCGCGGCCTGCCGATGGCTGACA
TGAGGCGCAACCTGCAGCGGATGATAGACCTGGCCCAGGCGCGCAAGGCCA
AGGTGCTGCTGGTGGGCATGGCGCTGCCACCCAACTATGGCCCCCGCTACGG
CGCCGAGTTCCGCGCCGTTTATGACGATTTGGCCCGCCGCAACCGCCTGGCCT
ACGTGCCGCTGCTGGTCGAGGGCTTCGCCGGCGACCTCGGCGCCTTCCAGCC
CGACGGCCTGCATCCCCGCGCGGAGAAGCAGGCCACCATGATGCGCACGGTC
AAGGCAAAACTGCCAGTGAAATAA (SEQ ID NO:100)

Chromobacterium violaceum Cvi (Q7NRP5) (Amino Acid)
MRSIVCKMLFPLLLLWQLPALAATVLVFGDSLSAGYGLAPGQGWAALLARDLSP
RHKVVNASVSGETSAGGLSRLPDALARHQPDVLVLELGANDGLRGLPMADMRR

NLQRMIDLAQARKAKVLLVGMALPPNYGPRYGAEFRAVYDDLARRNRLAYVPL
LVEGFAGDLGAFQPDGLHPRAEKQATMMRTVKAKLPVK (SEQ ID NO:101)

Vibrio vulnificus Vvu (AA007232) (DNA)
ATGTTTTTCCTTTCTAGCGTCGCACACGCAACCGAGAAAGTGTTAATTCTTGG
CGACAGCCTAAGTGCAGGATACAACATGTCTGCAGAGCAGGCTTGGCCTAAT
TTGTTACCAGAAGCATTGAATACATACGGAAAAAACGTAGAAGTGATCAACG
CCAGTATCTCTGGAGACACAACCGGCAATGGACTATCTCGTCTGCCTGAGTTG
TTAAAAACGCACTCACCAGACTGGGTGCTTATTGAGTTGGGTGCCAATGATG
GCTTGCGAGGTTTCCCGCATAAAGTGATCTCTTCAAACCTTTCGCGAATGATT
CAACTCAGTAAAGCCTCAGACGCTAAAGTCGCATTGATGCAAATTCGTGTAC
CGCCTAACTATGGCAAGCGCTACACCGATGCATTTGTCGAACTCTACCCTACG
CTTGCTGAACATCACCAAGTCCCGTTGCTCCCCTTTTTCTTAGAGGAAGTGAT
CGTGAAACCGGAATGGATGATGCCTGATGGCTTACACCCAATGCCCGAAGCT
CAGCCTTGGATCGCTCAATTTGTTGCAAAAACGTTTTACAAACATCTCTAA
(SEQ ID NO:102)

Vibrio vulnificus Vvu (AA007232) (Amino Acid)
MFFLSSVAHATEKVLILGDSLSAGYNMSAEQAWPNLLPEALNTYGKNVEVINASI
SGDTTGNGLSRLPELLKTHSPDWVLIELGANDGLRGFPHKVISSNLSRMIQLSKAS
DAKVALMQIRVPPNYGKRYTDAFVELYPTLAEHHQVPLLPFFLEEVIVKPEWMM
PDGLHPMPEAQPWIAQFVAKTFYKHL (SEQ ID NO:103)

Ralstonia eutropha Reu (ZP00166901) (DNA)
ATGCCATTGACCGCGCCGTCTGAAGTCGATCCGCTGCAAATCCTGGTCTATGC
CGATTCGCTTTCGTGGGGCATCGTGCCCGGCACCCGCCGGCGGCTTCCCTTCC
CGGTTCGCTGGCCAGGCCGGCTCGAACTCGGCCTGAACGCCGACGGCGGCGC
CCCGGTCCGCATCATCGAGGACTGCCTGAACGGCCGGCGCACCGTCTGGGAC
GACCCATTCAAACCGGGCCGCAACGGCTTGCAAGGGCTGGCGCAGCGCATCG
AGATCCATTCCCCGGTGGCGCTCGTGGTTTTGATGCTGGGCAACAACGATTTC
CAGTCCATGCATCCGCACAACGCCTGGCATGCGGCACAGGGCGTCGGCGCGC
TGGTCCACGCCATCCGGACGGCGCCGATCGAACCGGGAATGCCGGTGCCGCC
GATCCTGGTGGTGGTGCCGCCGCCGATCCGCACGCCCTGCGGGCCGCTCGCG
CCCAAGTTCGCCGGCGGCGAACACAAGTGGGCAGGCCTGCCCGAGGCGCTGC
GCGAACTGTGCGCCACTGTCGACTGCTCGCTGTTCGATGCGGGTACCGTGATC
CAGAGCAGTGCCGTCGACGGCGTACACCTTGACGCCGATGCCCATGTCGCCC

TGGGCGATGCCCTGCAACCGGTCGTTCGTGCGCTGCTCGCCGAATCCTCGGG
ACATCCCTCCTAA (SEQ ID NO:104)

Ralstonia eutropha Reu (ZP00166901) (Amino Acid)
MPLTAPSEVDPLQILVYADSLSWGIVPGTRRRLPFPVRWPGRLELGLNADGGAPV
RIIEDCLNGRRTVWDDPFKPGRNGLQGLAQRIEIHSPVALVVLMLGNNDFQSMHP
HNAWHAAQGVGALVHAIRTAPIEPGMPVPPILVVVPPPIRTPCGPLAPKFAGGEH
KWAGLPEALRELCATVDCSLFDAGTVIQSSAVDGVHLDADAHVALGDALQPVV
RALLAESSGHPS (SEQ ID NO:105)

Salmonella typhimurium Stm (AAC38796) (DNA)
ATGACCCAAAAGCGTACCCTGCTAAAATACGGCATACTCTCGCTGGCGCTGG
CCGCGCCATTATCTGCCTGTGCGTTTGACTCTCTTACGGTGATTGGCGATAGC
CTTAGCGATACCGGTAATAACGGTCGCTGGACCTGGGATAGTGGTCAAAATA
AGCTCTACGACGAACAGTTGGCCGAACGATATGGGCTGGAATTAAGCCCTTC
CAGCAATGGCGGCTCTAATTATGCCGCCGGCGGCGCGACGGCGACCCCGGAA
TTAAACCCGCAGGATAATACCGCGGATCAGGTACGGCAGTGGCTTGCCAAAA
CGGGGGGAAAAGCCGACCACAACGGTTTGTATATTCACTGGGTCGGCGGAAA
CGATCTGGCGGCGGCCATCGCGCAACCAACCATGGCACAGCAAATAGCCGGT
AATAGCGCCACTAGCGCGGCGGCGCAGGTAGGGCTGTTACTGGATGCCGGCG
CCGGGCTGGTCGTGGTGCCAAACGTACCGGATATTAGTGCGACGCCAATGCT
TCTGGAGGCGGTAATCACCGCTGGGCTGGGCGCAGCGGCGCCCCCGGCGCTA
AAAGCGGCGTTAGATGCGCTGGCGGAGGGCGCTACGCCCGATTTCGCCAGTC
GGCAACAGGCGATCCGCAAGGCGCTGCTGGCGGCGGCTGCAACGGTAAGCA
GCAATCCATTTATTCAGCAACTGCTCGTTGAACAACTGCTGGCGGGCTATGAA
GCGGCGGCAGGGCAGGCGTCAGCTCTGACCGATTATTATAATCAGATGGAAG
AGAAGGGGCTGGAGCAACACGGCGGCAATATAGCCCGTGCCGATATCAACG
GCCTCTTTAAGGAAATTCTTGCCAACCCGCAGGCGTTTGGTCTGACAAATACC
GTAGGTATGGCCTGCCCGCCTGGCGTATCCGCTTCGGCGTGCTCCTCGGCAAT
GCCTGGATTTAATGCGTCGCAGGACTATGTGTTTGCCGATCATTTACATCCCG
GTCCGCAGGTCCATACCATTATTGCGCAATATATTCAGTCGATCATTGCCGCG
CCGGTACAGGCGACATACCTGAACCAAAGCGTTCAGTCGATGGCGCAAGGCA
GTCGTACCACGCTTGACAGCCGTTATCAGCAGCTTCGCCAGGGGGAAAATCC
TGTTGGTTCGCTGGGCATGTTCGGCGGATACAGCGGGGGATATCAACGTTAT
GATAATAATGAGGCCGACGGGAACGGTAATCATAATAATCTGACGGTTGGCG
TCGATTATCAGCTTAACGAGCAGGTTCTGCTGGGAGGGCTGATAGCCGGTTCT
CTGGATAAGCAACATCCTGACGATAATTATCGTTATGATGCCCGCGGTTTTCA

GGCCGCCGTATTCAGCCATTTACGCGCCGGTCAGGCGTGGCTGGATAGCGAT
TTACACTTTCTGTCCGCTAAATTCAGTAACATTCAGCGCAGTATAACGCTCGG
TGCGCTAAGACGGGTGGAAGAGGGCGAAACCAACGGTCGGCTGTCGGGCGC
GAGCTTAACCAGCGGTTATGATTTTGTCATGGTGCCGTGGTTAACGACCGGAC
CGATGCTGCAATATGCATGGGATTACAGCCACGTTAATGGTTATAGCGAGAA
GCTCAATACCAGTACATCAATGCGTTTTGGTGACCAAAACGCCCATTCGCAG
GTGGGTAGCGCGGGTTGGCGTCTGGATCTTCGCCACAGCATCATTCACTCCTG
GGCGCAGATTAATTATCGCCGTCAGTTTGGCGATGATACGTATGTGGCGAAC
GGCGGCCTTAAATCGACCGCGCTGACGTTTAGCCGCGACGGAAAAACGCAGG
ATAAAAACTGGGTTGATATCGCGATTGGCGCAGATTTTCCGCTGTCGGCAAC
GGTGTCCGCTTTCGCCGGGCTGTCGCAAACGGCAGGGTTAAGCGATGGCAAT
CAAACCCGTTATAACGTTGGGTTTAGCGCCCGATTTTAA (SEQ ID NO:106)

Salmonella typhimurium Stm (AAC38796) (Amino Acid)
MTQKRTLLKYGILSLALAAPLSACAFDSLTVIGDSLSDTGNNGRWTWDSGQNKL
YDEQLAERYGLELSPSSNGGSNYAAGGATATPELNPQDNTADQVRQWLAKTGG
KADHNGLYIHWVGGNDLAAAIAQPTMAQQIAGNSATSAAAQVGLLLDAGAGLV
VVPNVPDISATPMLLEAVITAGLGAAAPPALKAALDALAEGATPDFASRQQAIRK
ALLAAAATVSSNPFIQQLLVEQLLAGYEAAAGQASALTDYYNQMEEKGLEQHG
GNIARADINGLFKEILANPQAFGLTNTVGMACPPGVSASACSSAMPGFNASQDYV
FADHLHPGPQVHTIIAQYIQSIIAAPVQATYLNQSVQSMAQGSRTTLDSRYQQLRQ
GENPVGSLGMFGGYSGGYQRYDNNEADGNGNHNNLTVGVDYQLNEQVLLGGLI
AGSLDKQHPDDNYRYDARGFQAAVFSHLRAGQAWLDSDLHFLSAKFSNIQRSIT
LGALRRVEEGETNGRLSGASLTSGYDFVMVPWLTTGPMLQYAWDYSHVNGYSE
KLNTSTSMRFGDQNAHSQVGSAGWRLDLRHSIIHSWAQINYRRQFGDDTYVAN
GGLKSTALTFSRDGKTQDKNWVDIAIGADFPLSATVSAFAGLSQTAGLSDGNQTR
YNVGFSARF (SEQ ID NO:107)

In total, nine of the new "GDSL"-type esterases were identified in 6 metagenomic libraries and BRAIN's esterase/lipase library. Eight of these genes were heterologously expressed in *E. coli* and the resulting enzymes analyzed for activity in the assays described herein. The characterization of these enzymes for perhydrolase activity revealed that one displayed the desired activity. A second one was predicted to show this activity due to the presence of amino acids conserved among this group of enzymes.

Comparison of the sequences of enzymes for which the presence or absence of the desired perhydrolase activity was determined led to the identification of 19 amino acid positions which were conserved among the enzymes which displayed the desired perhydrolase activity. Thus, it is contemplated that these conserved amino acids are essential for the perhydrolase reaction and/or is a structural feature of perhydrolase enzymes.

One of the identified structural motifs ("G/ARTT") conserved among esterases with the desired perhydrolase activity was used to design degenerate primers which provided the means to focus the screening on true perhydrolases among "GDSL"-type esterases. Indeed, the use of these "G/ARTT" primers led to the identification of enzymes with the desired perhydrolase activity from the metagenome. However, it is not intended that the use of the metagenome be limited to any particular assay method. Indeed, it is contemplated that the metagenome be searched by assaying for a particular enzyme activity or activities desired (*e.g.*, perhydrolysis and/or acyltransferase (cofactor dependent or independent) activity). In addition, screening using poly and/or monoclonal anti-sera directed against a protein of interest finds use in the present invention. In additional embodiments, the metagenome is searched using degenerate primer sets based on the sequence of the protein of interest.

In addition, the knowledge of the structure/function relationship of perhydrolases allowed searching for these enzymes in genome sequences of cultivable microorganisms. Of 16 "GDSL"-type esterases identified in different bacterial isolates, the corresponding genes of 10 enzymes were amplified and heterologously expressed in *E. coli*. The resulting enzyme samples of seven clones were analyzed using the assays described herein. Of five samples characterized to date, 4 enzymes indeed showed the desired activity and all results confirmed the proposed relationship between primary structural determinants and the function of perhydrolases. Thus, an enzyme library of 19 "GDSL"-type esterases comprising at least 6 perhydrolases with the desired perhydrolase activity was set up. The identified correlation between the structure and function of perhydrolases provides a definition of the sequence space used by enzymes with the desired perhydrolase activity.

Comparisons were made of protein sequences of enzymes for which the absence or presence of the desired perhydrolase activity. This revealed a correlation between the presence of certain amino acids and the capability to perform perhydrolase reactions. This knowledge was used to identify enzymes containing these conserved amino acids in sequenced genomes from cultivable microorganisms. The following enzymes were identified and experiments to amplify the genes from the genomic DNA of the corresponding strains using specific primers were performed.

Table 1. "GDSL"-type Esterases with a "GRTT"-Motif From Bacterial Isolates

Isolate	Protein Identifier	Acronym	Amplicon	Expression Vector
Sinorhizobium meliloti	Sma1993	Sme I	yes	pLO_SmeI
Sinorhizobium meliloti	Q92XZ1	Sme II	yes	pET26_SmeII
Sinorhizobium meliloti	Q9EV56	Sme III	yes	pET26_SmeIII
Agrobacterium rhizogenes	Q9KWB1	Arh I	no	-
Agrobacterium rhizogenes	Q9KWA6	Arh II	no	-

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Agrobacterium tumefaciens	AAD02335	Atu III	yes	pET26_AtuIII
Mesorhizobium loti	Q98MY5	Mlo I	yes	pET26_Mlo
Mesorhizobium loti	ZP_00197751	Mlo II	no	-
Ralstonia solanacearum	Q8XQI0	Rso	no	-
Ralstonia eutropha	ZP_00166901	Reu	yes	n.d.
Moraxella bovis	AAK53448	Mbo	yes	pET26_Mbo
Burkholderia cepacia	ZP_00216984	Bce	no	-
Chromobacterium violaceum	Q7NRP5	Cvi	yes	pET26_Cvi
Pirellula sp.	NP_865746	Psp	n.d.	n.d.
Vibrio vulnificus	AA007232	Vvu	yes	pET26_Vvu
Salmonella typhimurium	AAC38796	Sty	yes	pET26_Sty

In the cases of *A. rhizogenes, M. loti* (enzyme II), *R. solanacearum* and *B. cepacia* no amplicon could be generated. It was thought that this was probably due to genetic differences between the strains used in this investigation and those used for the sequencing of the genes deposited in the public domain databases. One reason might be that the corresponding genes are located on plasmids which are not present in the strains used in this investigation. However, it is not intended that the present invention be limited to any particular mechanism or theory.

The amplicons from all other strains were sequenced. In many cases there were differences between the sequence from the databases and the sequence determined during the development of the present invention. By sequencing two clones from independent amplifications, mutations introduced by the polymerase could be nearly excluded. The sequences of the genes and the deduced amino acid sequences of "GDSL"-type esterases with a "GRTT"-motif or variations from bacterial isolates are provided below:

SMa1993_*Sinorhizobium meliloti* (Sme I) (SEQ ID NOS:88 and 89)
Q92XZ1_*Sinorhizobium meliloti* (Sme II) (SEQ ID NOS:90 and 91)
Q9EV56_*Sinorhizobium meliloti* (Sme III) (SEQ ID NOS:92 and 93)
AAD02335_*Agrobacterium tumefaciens* (Atu III) (SEQ ID NOS: 94 and 95)
Q98MY5_*Mesorhizobium loti* (Mlo I) (SEQ ID NOS:96 and 97)
ZP_00166901_*Ralstonia eutropha* (Reu) (SEQ ID NOS:104 and 105)
AAK53448_*Moraxella bovis* (Mbo) (SEQ ID NOS: 98 and 99)
Q7NRP5_*Chromobacterium violaceum* (Cvi) (SEQ ID NOS:100 and 101)
AA007232_*Vibrio vulnificus* (Vvu) (SEQ ID NOS:102 and 103)
AAC38796_*Salmonella typhimurium* (Stm) (SEQ ID NOS:106 and 107)

Q9KWB1_ ***Agrobacterium rhizogenes*** **(Arh I)**
MICHKGGEEMRSVLCYGDSNTHGQIPGGSPLDRYGPNERWPGVLRRELGSQWY
VIEEGLSGRTTVRDDPIEGTMKNGRTYLRPCLMSHAILDLVIIMLGTNDLKARFGQ
PPSEVAMGIGCLVYDIRELAPGPGGKPPEIMVVAPPPMLDDIKEWEPIFSGAQEKS
RRLALEFEIIADSLEVHFFDAATVASCDPCDGFHINREAHEALGTALAREVEAIGW
R (SEQ ID NO:108)

ATGATTTGCCATAAAGGTGGGGAGGAAATGCGGTCAGTCTTATGCTACGGCG
ACTCGAATACGCACGGCCAGATTCCGGGGGGCTCACCGCTCGACCGATACGG
GCCGAACGAGCGCTGGCCTGGCGTTTTGAGACGGGAGCTTGGAAGCCAGTGG
TATGTGATCGAGGAGGGCCTGAGTGGCCGCACGACGGTTCGCGACGATCCGA
TCGAGGGCACGATGAAAAACGGCCGGACCTACCTGCGTCCGTGCCTCATGAG
CCACGCGATCCTCGATCTCGTGATTATCATGCTCGGGACGAACGACCTGAAA
GCGCGCTTCGGTCAACCGCCATCGGAAGTGGCGATGGGGATCGGCTGCCTCG
TCTACGATATCAGGGAGCTGGCGCCCGGACCGGGCGGCAAGCCCCCCGAAAT
CATGGTGGTTGCTCCGCCGCCGATGCTGGACGATATCAAGGAATGGGAACCC

ATATTTTCCGGCGCCCAGGAGAAATCCCGGCGTCTCGCGCTTGAGTTTGAAAT
TATTGCTGATTCGCTTGAAGTACACTTCTTTGACGCCGCGACCGTCGCATCGT
GTGATCCTTGCGATGGTTTTCACATCAACCGGGAAGCGCATGAAGCCTTGGG
AACAGCGCTTGCCAGGGAAGTGGAGGCGATCGGTTGGAGATGATGA (SEQ ID NO:109)

Q9KWA6_*Agrobacterium rhizogenes* (Arh II)
MAESRSILCFGDSLTWGWIPVPESSPTLRYPFEQRWTGAMAAALGDGYSIIEEGLS
ARTTSVEDPNDPRLNGSAYLPMALASHLPLDLVIILLGTNDTKSYFRRTPYEIANG
MGKLAGQVLTSAGGIGTPYPAPKLLIVSPPPLAPMPDPWFEGMFGGGYEKSLELA
KQYKALANFLKVDFLDAGEFVKTDGCDGIHFSAETNITLGHAIAAKVEAIFSQEA
KNAAA (SEQ ID NO:110)

ATGGCAGAGAGCCGCTCAATATTATGTTTTGGGGATTCACTCACATGGGGTTG
GATTCCGGTACCGGAGTCGTCGCCGACGCTCAGATATCCCTTTGAGCAGCGCT
GGACCGGTGCAATGGCTGCGGCACTCGGTGACGGCTATTCAATCATCGAGGA
AGGCCTTTCCGCCCGCACGACCAGCGTCGAGGATCCGAACGATCCCAGGCTG
AACGGCAGCGCCTACCTGCCGATGGCGCTCGCCAGCCATCTGCCGCTCGATC
TCGTCATCATCCTTCTCGGCACCAACGACACCAAGTCCTATTTCCGCCGCACG
CCCTATGAGATCGCCAACGGCATGGGCAAGCTTGCCGGACAGGTTCTGACCT
CGGCCGGCGGGATCGGCACGCCCTACCCTGCCCCGAAGCTTCTGATCGTTTC
GCCGCCGCCGCTCGCTCCCATGCCTGACCCGTGGTTCGAAGGCATGTTCGGTG
GCGGTTACGAAAAGTCGCTCGAACTCGCAAAGCAGTACAAGGCGCTCGCCAA
CTTCCTGAAGGTCGACTTCCTCGACGCCGGCGAGTTTGTAAAGACCGACGGC
TGCGATGGAATCCATTTCTCCGCCGAGACGAACATCACGCTCGGCCATGCGA
TCGCGGCGAAGGTCGAAGCGATTTTCTCACAAGAGGCGAAGAACGCTGCGGC
TTAG (SEQ ID NO:111)

ZP_00197751_*Mesorhizobium loti* (Mlo II)
MKTILCYGDSLTWGYDAVGPSRHAYEDRWPSVLQGRLGSSARVIAEGLCGRTTA
FDDWVAGADRNGARILPTLLATHSPLDLVIVMLGTNDMKSFVCGRAIGAKQGME
RIVQIIRGQPYSFNYKVPSILLVAPPPLCATENSDFAEIFEGGMAESQKLAPLYAAL
AQQTGCAFFDAGTVARTTPLDGIHLDAENTRAIGAGLEPVVRQALGL (SEQ ID NO:112)

ATGAAGACCATCCTTTGTTACGGTGACTCCCTCACTTGGGGCTATGATGCCGT
CGGACCCATGAAGACCATCCTTTGTTACGGTGACTCCCTCACTTGGGGCTATG
ATGCCGTCGGACCCTCACGGCATGCTTATGAGGATCGATGGCCCTCCGTACTG

CAAGGCCGCCTCGGTAGCAGTGCGCGGGTGATCGCCGAGGGGCTTTGCGGCC
GCACAACTGCGTTTGACGACTGGGTCGCTGGTGCGGACCGGAACGGTGCGCG
CATCCTGCCGACGCTTCTTGCGACCCATTCACCGCTTGACCTCGTTATCGTCA
TGCTCGGGACGAACGACATGAAATCGTTCGTTTGCGGGCGCGCTATCGGCGC
CAAGCAGGGGATGGAGCGGATCGTCCAGATCATCCGCGGGCAGCCTTATTCC
TTCAATTATAAGGTACCGTCGATTCTTCTCGTGGCGCCGCCGCCGCTGTGCGC
TACCGAAAACAGCGATTTCGCGGAAATTTTTGAAGGTGGCATGGCTGAATCG
CAAAAGCTCGCGCCGCTTTATGCCGCGCTGGCCCAGCAAACCGGATGCGCCT
TCTTCGATGCAGGCACTGTGGCCCGCACGACACCGCTCGACGGTATTCACCTC
GATGCTGAAAACACGCGCGCCATTGGTGCCGGCCTGGAGCCGGTGGTCCGCC
AAGCGCTTGGATTGTGA (SEQ ID NO:113)

Q8XQI0_*Ralstonia solanacearum* (Rso)
MQQILLYSDSLSWGIIPGTRRRLPFAARWAGVMEHALQAQGHAVRIVEDCLNGR
TTVLDDPARPGRNGLQGLAQRIEAHAPLALVILMLGTNDFQAIFRHTAQDAAQG
VAQLVRAIRQAPIEPGMPVPPVLIVVPPAITAPAGAMADKFADAQPKCAGLAQAY
RATAQTLGCHVFDANSVTPASRVDGIHLDADQHAQLGRAMAQVVGTLLAQ
(SEQ ID NO:114)

ATGCAACAGATCCTGCTCTATTCCGACTCGCTCTCCTGGGGCATCATCCCCGG
CACCCGCCGGCGCCTGCCGTTCGCCGCCCGCTGGGCCGGGGTCATGGAACAC
GCGCTGCAGGCGCAAGGGCACGCCGTGCGCATCGTCGAAGACTGCCTCAATG
GACGCACCACGGTGCTCGACGATCCCGCGCGGCCGGGGCGCAACGGACTGCA
GGGGCTCGCGCAGCGGATCGAAGCGCACGCCCCGCTTGCCCTGGTCATCCTG
ATGCTCGGCACCAACGACTTCCAGGCGATCTTCCGGCACACCGCCCAGGACG
CGGCGCAAGGCGTGGCGCAGCTGGTGCGGGCCATCCGCCAGGCGCCGATCGA
ACCCGGCATGCCGGTGCCGCCCGTGCTGATCGTGGTGCCGCCGGCCATCACC
GCGCCGGCCGGGGCGATGGCCGACAAGTTTGCCGACGCGCAGCCCAAGTGCG
CCGGCCTTGCGCAGGCCTATCGGGCAACGGCGCAAACGCTAGGCTGCCACGT
CTTCGATGCGAACAGCGTCACGCCGGCCAGCCGCGTGGACGGCATCCACCTC
GATGCCGACCAGCATGCGCAGCTGGGCCGGGCGATGGCGCAGGTCGTCGGG
ACGCTGCTTGCGCAATAA (SEQ ID NO:115)

ZP_00216984 *Burkholderia cepacia* (Bce)
ATGACGATGACGCAGAAAACCGTGCTCTGCTACGGCGATTCGAACACGCATG
GCACACGCCCGATGACGCATGCTGGCGGACTGGGGCGGTTTGCACGCGAAGA
ACGCTGGACCGGCGTGCTGGCGCAAACGCTCGGTGCGAGCTGGCGGGTCATT
GAAGAAGGGTTGCCCGCGCGTACGACCGTGCATGACGATCCGATCGAAGGCC

GGCACAAGAATGGTTTGTCGTATCTGCGCGCGTGCGTCGAAAGCCACTTGCC
CGTCGATGTCGTCGTGCTGATGCTCGGGACCAACGATCTGAAGACACGCTTCT
CGGTCACGCCCGCCGACATCGCGACATCGGTCGGCGTATTGCTTGCCAAGAT
CGCTGCGTGCGGCGCCGGTCCGTCCGGTGCGTCACCGAAGCTCGTGCTGATG
GCGCCTGCGCCGATCGTCGAGGTCGGATTCCTCGGCGAGATCTTTGCGGGCG
GCGCAGCGAAGTCGCGGCAGCTCGCGAAGCGGTACGAACAGGTGGCAAGCG
ATGCCGGTGCGCACTTTCTCGATGCCGGCGCGATCGTCGAGGTGAGCCCGGT
GGATGGCGTTCACTTCGCGGCCGATCAGCATCGTGTGCTCGGGCAGCGGGTC
GCTGCCCTTCTGCAGCAGATTGCGTAA (SEQ ID NO:116)

MTMTQKTVLCYGDSNTHGTRPMTHAGGLGRFAREERWTGVLAQTLGASWRVI
EEGLPARTTVHDDPIEGRHKNGLSYLRACVESHLPVDVVVLMLGTNDLKTRFSV
TPADIATSVGVLLAKIAACGAGPSGASPKLVLMAPAPIVEVGFLGEIFAGGAAKSR
QLAKRYEQVASDAGAHFLDAGAIVEVSPVDGVHFAADQHRVLGQRVAALLQQI
A (SEQ ID NO:117)

NP_865746 *Pirellula sp* (Psp)
MHSILIYGDSLSWGIIPGTRRRFAFHQRWPGVMEIELRQTGIDARVIEDCLNGRRT
VLEDPIKPGRNGLDGLQQRIEINSPLSLVVLFLGTNDFQSVHEFHAEQSAQGLALL
VDAIRRSPFEPGMPTPKILLVAPPTVHHPKLDMAAKFQNAETKSTGLADAIRKVS
TEHSCEFFDAATVTTTSVVDGVHLDQEQHQALGTALASTIAEILADC (SEQ ID
NO:118)

ATGCATTCAATCCTCATCTATGGCGATTCTCTCAGTTGGGGAATCATTCCCGG
CACGCGTCGTCGCTTCGCGTTCCATCAGCGTTGGCCGGGCGTCATGGAGATTG
AACTGCGACAAACTGGAATCGATGCCCGCGTCATCGAAGACTGCCTCAATGG
CCGACGAACCGTCTTGGAAGATCCAATCAAACCCGGACGCAATGGCCTGGAT
GGTTTGCAGCAACGGATCGAAATCAATTCACCTCTGTCACTGGTCGTGCTCTT
TCTGGGGACCAACGATTTCCAGTCCGTCCACGAATTCCATGCCGAGCAATCG
GCACAAGGACTCGCACTGCTTGTCGACGCCATTCGTCGCTCCCCTTTCGAACC
AGGAATGCCGACACCGAAAATCCTGCTTGTCGCACCACCGACGGTTCACCAC
CCGAAACTTGATATGGCGGCGAAGTTCCAAAACGCGGAAACGAAATCGACG
GGACTCGCAGATGCGATTCGCAAGGTCTCAACAGAACACTCCTGCGAATTCT
TCGATGCGGCCACGGTCACCACAACAAGTGTCGTCGACGGAGTCCATCTCGA
TCAAGAACAACATCAAGCACTCGGTACCGCACTGGCATCGACAATCGCTGAA
ATACTAGCAGACTGTTGA (SEQ ID NO:119)

As indicated above, the above sequences are the protein sequences and the coding sequences of "GDSL-type" esterases with a "GRTT"-motif or similar motifs from different bacterial isolates. The DNA sequences represent the target-DNA from which specific primers were deduced. All amplicons were ligated as *NdeI/XhoI*-fragments to pET26 thereby eliminating the *pel*B-leader sequence of this vector. All of the "GDSL-type" esterases from these isolates were expressed in *E. coli* Rosetta (DE3) at 28°C. The expression was induced by addition of 100 µM IPTG at an $O.D._{580} = 1$ and the cells were harvested 20 h after induction. Only the cells expressing the enzymes from *M. bovis* and *S. typhimurium* were collected 4 h after induction, since previous experiments had shown that the highest activity could be obtained at this point of time. Table 2 summarizes the expression experiments.

Table 2: Expression and Characterization of "GDSL"-type Esterases From Bacterial Isolates for Perhydrolase Activity

Strain	Enzyme	Expression Level[2]	Solubility[3]	Activity[4]	Perhydrolase Activity	GRTT -Motif
S. meliloti	Sme I	+++	++	5770,0	yes	ARTT
S. meliloti	Sme II	+++	+++	85,0	yes	GRTT
S. meliloti	Sme III	+++	++	746,5	n.d.	GRTT
A. tumefaciens	Atu III	n.d[5].	n.d.	n.d.	n.d.	GRTT
M. loti	Mlo I	+++	++	1187,3	yes	GRTT
M. bovis[1]	Mbo	+	n.d.	25,2	yes	ARTT
C. violaceum	Cvi	+	+	2422,7	n.d.	GETS
V vulnificus	Vvu	n.d.	n.d.	n.d.	n.d.	GDTT
R. eutropha	Reu	n.d.	n.d.	n.d.	n.d.	GRRT
S. typhimurium[1]	Sty	+	n.d.	17,2	no	SRTT

[1] outer membrane localized autotransporter protein

[2] expression level: + moderate overexpression; ++ strong overexpression; +++ very strong overexpression as judged from SDS-PAGE-analysis

[3] as judged by SDS-PAGE-analysis

[4] towards *p*-nitrophenyl butyrate

[6] not determined

With the exception of the enzyme from *S. typhimurium,* all other enzymes tested showed the desired perhydrolase activity, confirming the correlation between the presence of certain conserved amino acids an the capability to perform perhydrolase reactions. Although the enzyme from *S. typhimurium* contains the GRTT-motif, it is different from the other enzymes by the location of this motif downstream from block V. In all other enzymes, this motif is located between block I and III, indicating that it might have a different function in the enzyme from *S. typhimurium.* Thus, the absence of perhydrolase activity in the enzyme from *S. typhimurium* also supports the identified structure/function-relationship of the perhydrolases provided by the present invention.

Screening of New "GDSL-type" Esterases in Metagenome Libraries

i) Library S279

The full-length sequence of the gene from clone M75bA2 was completed, as provided below.

```
  1  tgggcggttt cgcggagtcg agcagggaga gatgctcctg ggtcgtacga gttggtacgg
        g  r  f   r  g  v   e  q  g   e  m  l  l   g  r  t   s  w  y
 61  aggcatcgtt gaagatctca cgcctgcttg aatgcgcgcg gatatggaac ggaccggccg
     g  g  i  v   e  d  l   t  p  a   -  m  r  a   d  m  e   r  t  g
121  cgctggcgat cggtgtcggc gtggggctgg cgagcctgag cccggtcgcg ctggcgacgc
```

```
     r  a  g  d   r  c  r   r  g  a   g  e  p  e   p  g  r   a  g  d
181  cgccgcgggg caccgtgccg gtgttcaccc gatcggggac agcctgacgg acgagtattt
     a  a  a  g   h  r  a   g  v  h   p  i  q  d   s  l  t   d  e  y
241  tgagccgttc ttccagtggg ggttctgcgg gaagtcgtgg gccgagattt tggtggagac
     f  e  p  f   f  q  w   g  f  c   g  k  s  w   a  e  i   l  v  e
301  ggggcgggcg agcatgggcc cgacggcgca gcaggcgggg atcagcgagc cggagggatg
     t  g  r  a   s  m  g   p  t  a   q  q  a  g   i  s  e   p  e  g
361  gtcggatccg cggaacacgg ggtatcagca caactgggcg cggtactcgt ggagctcctc
     w  s  d  p   r  n  t   g  y  q   h  n  w  a   r  y  s   w  s  s
421  agacgcgctg accgaggagt cgccgggggc gacgctgagc gtgctgcttg ggcggagta
     s  d  a  l   t  e  e   s  p  g   a  t  l  s   v  l  l   g  a  e
481  cgcggtggtg ttcattggga ccaacgactt caatccgtcg tggccggcgt atcagagcgt
     y  a  v  v   f  i  q   t  n  d   f  n  p  s   w  p  a   y  q  s
541  gtatctgagc cagtggagcg acgagcagat cgacacgtac gtgaacgggg tggtgcagaa
     v  y  l  s   q  w  s   d  e  q   i  d  t  y   v  n  g   v  v  q
601  catcgcgcag atggtggact cgctgaagtc ggtcggggcg aaggtggtgc ttgcgccgcc
     n  i  a  q   m  v  d   s  l  k   s  v  g  a   k  v  v   l  a  p
661  ggtggatttt cagttcgcgg ggttcctgcg gaactcatgc ccggatccga tgctgcgcga
     p  v  d  f   q  f  a   g  f  l   r  n  s  c   p  d  p   m  l  r
721  gcaggcgggt attctgacac ggaagtgcca cgaccgggtg cggtcgatgg cgcggcagaa
     e  q  a  g   i  l  t   r  k  c   h  d  r  v   r  s  m   a  r  q
781  gcacgtggtg ttcgtggaca tgtggcggct gaaccgcgat ttgttcggca acgggttcgc
     k  h  v  v   f  v  d   m  w  r   l  n  r  d   l  f  g   n  g  f
```

```
841  gatcagctac ggccttcgga acacggtgcg cgtgggggac tcggagatcg ggctgcaact
     a i s y g l r n t v r v g d s e i g l q
901  ggccgggctg acgggatcgg cggggctggt tccggacggg atccatccgc agcgggtggt
     l a g l t g s a g l v p d q i h p q r v
961  gcaggggatc tgggcgaatg cgttcatcgt gggtctgaac gcgcatgggg cgaacatcgc
     v q g i w a n a f i v g l n a h g a n i
1021 gcccatcggc gaggcggaga tgtgcgcgat gggggggtc gtgtacgggg gaacggacac
     a p i g e a e m c a m g g v v y g g t d
1081 gctggcgaac ttcctgccgc cggtcgcggg ctacgtggag gacttccgca acgcgggga
     t l a n f l p p v a g y v e d f r n a g
1141 cttcgtgtgc acggcggact tcaaccatga ccttggcgtg acgccgacgg acatcttcgc
     d f v c t a d f n h d l g v t p t d i f
1201 gttcatcaac gcgtggttca tgaatgatcc ctcggcgcgg atgagcaacc cggagcacac
     a f i n a w f m n d p s a r m s n p e h
1261 gcagatcgag gacatcttcg tgtttctgaa tctgtggctg gtggggtgct gaggcagagt
     t q i e d i f v f l n l w l v g c - g r
1321 gggaaggggg tcagcccact tcgcgcgtct ggaagaggat gacggcgacg gagaggaaga
     v g r g s a h f a r l e e d d g d g e e
```

In the sequence of S279_M75bA2 provided above (DNA, SEQ ID NO:80; and amino acid sequence, SEQ ID NO:81), the coding sequence running from position 104 through 1312 is shown on a grey background. Conserved structural motifs are shown underlined and in bold.

The derived amino acid sequence showed the highest homology to a hypothetical protein (Y17D7A.2) from *Caenorhabditis elegans* (BlastP2; swisspir), although with a very high E-value of 2.5 (*i.e.*, indicating a non-reliable hit). The fact that no esterase is among the homologous proteins identified by the BlastP2-analysis indicates that this enzyme is a rather unusual "GDSL-type" esterase. Furthermore, the enzyme is characterized by unusually long peptides between the N-terminus and the "GDSL"-motif and the "DXXH"-motif of block V (containing the active site aspartic acid and histidine) and the C-terminus. The very C-terminal sequence shows similarity to a membrane lipoprotein lipid attachment site. A corresponding signal sequence of lipoproteins was not identified. The gene encoding M75bA5 was amplified but no further efforts were taken for this enzyme since it did not have the conserved amino acids typical of the perhydrolase of the present invention.

ii) Library S248

The clone carrying the sequence-tag SP7_3j5h which could have been part of a gene encoding a "GDSL"-type esterase was identified (M31bA11), and the sequence was elongated. This facilitated the determination that this sequence did not encode a "GDSL-type" esterase, because block V could not be identified. The generation of this amplicon can be explained by an "unspecific" hybridization of primer 5h with the first mismatches at nucleotides 10, 14 and 15 from the 3'-terminus of the primer. The sequence showed the highest homology to a hypothetical protein (KO3E5.5) from *Caenorhabditis elegans* with an E-value of 1.6, indicating a non-reliable hit. The sequence-tag from clone S248_M31bA11 is provided below.

```
         ----------------------->
1  cggaattatc atgctgggtt ttaatgacca gcgcgagagg atcaacgaca acctcgatta
      r  n  y   h  a  g   f  -  -  p   a  r  e   d  q  r   q  p  r  l
       g  i  i   m  l  q   f  n  d   q  r  e  r   i  n  d   n  l  d
        e  l   s  c  w  v   l  m  t   s  a  r   g  s  t  t   t  s  i

61  ctgggacgcc taccactccg tcctgggcga gagacagttt tattccggca attccaagat
       l  g  r   l  p  l   r  p  g  r   e  t  v   l  f  r   q  f  q  d
```

```
     y  w  d  a   y  h  s   v  l  g   e  r  q  f   y  s  g   n  s  k
      t  g  t   p  t  t  p   s  w  a   r  d  s   f  i  p  a   i  p  r

121  gttcgtcccc atcaccaaga tcgcggtgaa ggcgcgcaag acccggttca ccaatcagat
       v  r  p   h  h  q   d  r  g  e   g  a  q   d  p  v   h  q  s  d
     m  f  v  p   i  t  k   i  a  v   k  a  r  k   t  r  f   t  n  q
      c  s  s   p  s  p  r   s  r  -   r  r  a   r  p  g  s   p  i  r
                                         ◄————————o——oo-o-o
181  ttttcctcag tccggccgca acgtcgatgt caccaccacg gacggcacac tcccccacgc
       f  s  s   v  r  p   q  r  r  c   h  h  h   g  r  h   t  p  p  r
     i  f  p  q   s  g  r   n  v  d   v  t  t  t   d  q  t   l  p  h
      f  f  l   s  p  a  a   t  s  m   s  p  p   r  t  a  h   s  p  t
         ooo_oo_ooo
241  caccatgtcc ctggtcgagc actacatccg ggcctgccgc ctgcgcaccc agatcgttcc
       h  h  v   p  g  r   a  l  h  p   g  l  p   p  a  h   p  d  r  s
     a  t  m  s   l  v  e   h  y  i   r  a  c  r   l  r  t   q  i  v
      p  p  c   p  w  s  s   t  t  s   g  p  a   a  c  a  p   r  s  f

301  ggccctgatc gttaacggcg attgcgaagg catgtacagc atctatgtcg gctggtcgaa
       g  p  d   r  -  r   r  l  r  r   h  v  q   h  l  c   r  l  v  e
     p  a  l  i   v  n  g   d  c  e   g  m  y  s   i  y  v   g  w  s
      r  p  -   s  l  t  a   i  a  k   a  c  t   a  s  m  s   a  g  r

361  aaccaccaag catgttgttt cacgtgaaac aaagccggtc gaaagcgacg gcatggaatt
       n  h  q   a  c  c   f  t  -  n   k  a  g   r  k  r   r  h  g  i
     k  t  t  k   h  v  v   s  r  e   t  k  p  v   e  s  d   g  m  e
      k  p  p   s  m  l  f   h  v  k   q  s  r   s  k  a  t   a  w  n

421  tcccgaactg ggcgaagccg acgacatcac cgaagaaacg cttgagtgtg gccttcccga
       s  r  t   g  r  s   r  r  h  h   r  r  n   a  -  v   w  p  s  r
     f  p  e  l   g  e  a   d  d  i   t  e  e  t   l  e  c   g  l  p
      f  p  n   w  a  k  p   t  t  s   p  k  k   r  l  s  v   a  f  p

481  catcgaattg atctcggacg ccgatcttct cgtccttcca ccagcgccga caacattcca
       h  r  i   d  l  g   r  r  s  s   r  p  s   t  s  a   d  n  i  p
     d  i  e  l   i  s  d   a  d  l   l  v  l  p   p  a  p   t  t  f
      t  s  n   -  s  r  t   p  i  f   s  s  f   h  q  r  r   q  h  s

541  aggcgcttga gatgggcggg ttcggtcacg atcttgcgcc gtggacaagg gcaaggtccg
       r  r  l   r  w  a   g  s  v  t   i  l  r   r  g  q   g  q  g  p
     q  g  a  -   d  g  r   v  r  s   r  s  c  a   v  d  k   g  k  v
      k  a  l   e  m  g  g   f  g  h   d  l  a   p  w  t  r   a  r  s

601  cagatgatcg acgaggcgcg atcaccgaga tgccgcgacg atctgtcgac gctatgtcac
       q  m  i   d  e  a   r  s  p  r   c  r  d   d  l  s   t  l  c  h
     r  r  -  s   t  r  r   d  h  r   d  a  a  t   i  c  r   r  y  v
      a  d  d   r  r  g  a   i  t  e   m  p  r   r  s  v  d   a  m  s
```

```
661  cagcgcatgt ccgacggtgg aatgcaagac aggtnggntn gatcgggg(SEQ ID NO:120)
       q  r  m   s  d  g   g  m  q  d   r  ?  ?   ?  s  g(SEQ ID NO:121)
     t  s  a  c   p  t  v   e  c  k   t  g  ?  ?   d  r (SEQ ID NO:122)
      p  a  h   v  r  r  w   n  a  r   q  ?  ?   ?  i  g(SEQ ID NO:123)
```

In the above sequence-tag of the clone S248_M31bA11, the primers 3j and 5h are indicated. Hybridization between primer and template is indicated by arrows, mismatches by open circles. Putative conserved structural motifs are indicated in bold and underlined.

Several further sequence-tags were generated using different primer pairs of the primers 2 and 5 but none turned out to encode a "GDSL"-type esterases. The screening of this library was completed.

iii) Library M091

The elongation of the amplicon SP3_1j5h, which was identified in the insert-DNA of clone M24dG12 proved that the corresponding sequence does not encode a "GDSL"-type esterase. Whereas the sequence encoding a putative block V (DGTHP; SEQ ID NO:124) was found, the corresponding sequence encoding block I was missing. The amplicon was generated due to an "unspecific" hybridization of primer 1j with the first mismatches at positions 5, 10, 11 and 12 from the 3'-terminus of the primer. The sequence-tag of clone M091_ M24dG12 s shown below:

```
  1  gcctgatggc ttcgagttcg tcgaattcac ctcgccccag cccggcgtgc tggaggcggt

       a  -  w   l  r  v   r  r  i  h   l  a  p   a  r  r   a  g  g  g
         p  d  g   f  e  f   v  e  f   t  s  p  q   p  g  v   l  e  a
         l  m   a  s  s  s   s  n  s   p  r  p   s  p  a  c   w  r  r

 61  gtttgaaaag ctgggtttca ccctggtcgc caagcaccgg tccaaggatg tggtgctgta

       v  -  k   a  g  f   h  p  g  r   q  a  p   v  q  g   c  g  a  v
      v  f  e  k   l  g  f   t  l  v   a  k  h  r   s  k  d   v  v  l
```

```
      c  l  k   s  w  v  s .p  w  s   p  s  t   g  p  r  m   w  c  c
121  ccgccagaac ggcatcaact tcatcctgaa ccgcgagccc cacagccagg ccgcctactt
       p  p  e   r  h  q   l  h  p  e   p  r  a   p  q  p   g  r  l  l

     y  r  q  n   g  i  n   f  i  l   n  r  e  p   h  s  q   a  a  y
      t  a  r   t  a  s  t   s  s  -   t  a  s   p  t  a  r   p  p  t
181  tggtgccgag catggcccct ccgcctgtgg cctggccttc cgtgtgaagg atgcgcataa
       w  c  r   a  w  p   l  r  l  w   p  g  l   p  c  e   g  c  a  -

     f  g  a  e   h  g  p   s  a  c   g  l  a  f   r  v  k   d  a  h
      l  v  p   s  m  a  p   p  p  v   a  w  p   s  v  -  r   m  r  i
241  ggcttataac cgcgcgctgg aactgggcgc ccagcccatc gagatcccca ccggccccat
       g  l  -   p  r  a   g  t  g  r   p  a  h   r  d  p   h  r  p  h

     k  a  y  n   r  a  l   e  l  g   a  q  p  i . e  i  p   t  g  p
      r  l  i   t  a  r  w   n  w  a   p  s  p   s  r  s  p   p  a  p
                                      oooooo---oooo--o-o----ooo----o
301  ggaactgcgc ctgcccgcca tcaagggcat tggcggcgcc gcctctgtat ttgatcgacc
       g  t  a   p  a  r   h  q  g  h   w  r  r   r  l  c   i  -  s  t

     m  e  l  r   l  p  a   i  k  g   i  g  g  a   a  s  v   f  d  r
      w  n  c   a  c  p  p   s  r  a   l  a  a   p  p  l  y   l  i  d
        →
361  gctttgaaga cggcaagtcc atctacgaca tcgacttcga gttcatcgaa ggcgtggacc
       a  l  k   t  a  s   p  s  t  t   s  t  s   s  s  s   k  a  w  t
      p  l  -  r   r  q  v   h  l  r   h  r  l  r   v  h  r   r  r  g
      r  f  e   d  g  k  s   i  y  d   i  d  f   e  f  i  e   g  v  d
421  gccgccccgc ggggcatggc ctgaacgaga tcgatcacct cacgcacaac gtgtaccggg
       a  a  p   r  g  m   a  -  t  r   s  i  t   s  r  t   t  c  t  g
      p  p  p  r   g  a  w   p  e  r -d  r  s  p   h  a  q   r  v  p
      r  r  p   a  g  h  q   l  n  e   i  d  h   l  t  h  n   v  y  r
481  gccgcatggg cttctgggcc aacttctacg aaaagctgtt caacttccgc gaaatccgct
       a  a  w   a  s  g   p  t  s  t   k  s  c   s  t  s   a  k  s  a
      g  p  h  g   l  l  g   q  l  l   r  k  a  v   q  l  p   r  n  p
      g  r  m   g  f  w  a   n  f  y   e  k  l   f  n  f  r   e  i  r
541  acttcgacat ccagggcgaa tacacgggcc tgacctccaa ggccatgacc gcgcccgacg
       t  s  t   s  r  a   n  t  r  a   -  p  p   r  p  -   p  r  p  t
      l  l  r  h   p  g  r   i  h  g   p  d  l  q   g  h  d   r  a  r
      y  f  d   i  q  g  e   y  t  g   l  t  s   k  a  m  t   a  p  d
601  gcaagattcg catcccgctg aacgaagagt ccaagcaggg cggcggccag atcgaagaat
       a  r  f   a  s  r   -  t  k  s   p  s  r   a  a  a   r  s  k  n
```

```
      r  q  d  s   h  p  a   e  r  r   v  q  a  g   r  r  p   d  r  r
       g  k  i   r  i  p  l   n  e  e   s  k  q   g  g  g  q   i  e  e
661   ttttgatgca attcaacggc gagggcattc agcacatcgc gctgatctgc gacaacctgc
        f  -  c   n  s  t   a  r  a  f   s  t  s  r  -  s   a  t  t  c

      i  f  d  a   i  q  r   r  g  h   s  a  h  r   a  d  l   r  q  p
       f  l  m   q  f  n  g   e  g  i   q  h  i   a  l  i  c   d  n  l
721   tggacgtggt ggacaagctg ggcatggccg gcgtgcagct ggccaccgcg cccaacgagg
        w  t  w  w  t  s   w  a  w  p   a  c  s   w  p  p   r  p  t  r

      a  g  r  g   g  q  a   g  h  g   r  r  a  a   g  h  r   a  q  r
       l  d  v   v  d  k  l   g  m  a   g  v  q   l  a  t  a   p  n  e

781   tctattacga aatgctggac acccgcctgc ccggccacgg ccagccggtg cccgagctgc
        s  i  t   k  c  w   t  p  a  c   p  a  t   a  s  r   c  p  s  c

      g  l  l  r   n  a  g   h  p  p   a  r  p  r   p  a  g   a  r  a
       v  y  y   e  m  l  d   t  r  l   p  g  h   g  q  p  v   p  e  l
                                        ◄────────────o-oo──oooo-o
841   agtcgcgcgg catcttgctg gacggcacca cggccgacgg cacgcacccg cctgctagct
        s  r  a   a  s  c   w  t  a  p   r  p  t   a  r  t   r  l  l  a

      a  v  a  r   h  l  a   g  r  h   h  g  r  r   h  a  p   a  c  -
       q  s  r   g  i  l  l   d  g  t   t  a  d   q  t  h  p   p  a  s
        ooo-o
901   tcagatcttc tccacgccca tgctgggccc ggtgttcttc gaattcatcc agcgcgaggg
        s  d  l   l  h  a   h  a  g  p   g  v  l   r  i  h   p  a  r  g

      l  q  i  f   s  t  p   m  l  g   p  v  f  f   e  f  i  q  r  e
       f  r  s   s  p  r  p   c  w  a   r  c  s   s  n  s  s   s  a  r
961   cgactaccgc gacggctttg gcgaaggcaa cttcaaggcg ctgttcgagt cgctggaacg
        r  l  p   r  r  l   w  r  r  q   l  q  g   a  v  r   v  a  g  t

      g  d  y  r   d  g  f   g  e  g   n  f  k  a   l  f  e   s  l  e
       a  t  t   a  t  a  l   a  k  a   t  s  r   r  c  s  s   r  w  n
1021  cgaccagatc cgccgtggtg tgctgaacac ataagacatc agacatccag ggttaaccct
        r  p  d   p  p  w   c  a  e  h   i  r  h   q  t  s   r  v  n  p

      r  d  q  i   r  r  g   v  l  n   t  -  d  i   r  h  p   g  l  t
       a  t  r   s  a  v  v   c  -  t   h  k  t   s  d  i  q   g  -  p
1081  gcacaggtgc ctatactgcg cgctccccgg aactcaaaag gatcccgatg tcgctccgta
```

```
        a  q  v   p  i  l   r  a  p  r   n  s  k   g  s  r   c  r  s  v

       l  h  r  c   l  y  c   a  l  p   g  t  q  k   d  p  d   v  a  p
        c  t  g   a  y  t  a   r  s  p   e  l  k   r  i  p  m   s  l  r

1141  gcaccctgtt cagcaccctt ttggccggcg cagccactgt cgcgctggcg cagaacccgt

        a  p  c   s  a  p   f  w  p  a   q  p  l   s  r  w   r  r  t  r

       -  h  p  v   q  h  p   f  g  r   r  s  h  c   r  a  g   a  e  p
        s  t  l   f  s  t  l   l  a  g   a  a  t   v  a  l  a   q  n  p

1201  ctgcccgctc acatcg  (SEQ ID NO:125)
        l  p  a   h  i   (SEQ ID NO:126)
      v  c  p  l   t  s  (SEQ ID NO:127)
       s  a  r   s  h     (SEQ ID NO:128)
```

Sequence-tag of the clone M091_M24dG12. The primers 1j and 5h are indicated in the above sequence-tag of the clone M091_M24dG12. Hybridization between primer and template is indicated by arrows, mismatches by open circles. Putative conserved structural motifs are depicted in bold and underlined.

A further sequence-tag (SP1_2b5h) was generated using the primer pair 2b/5h. A BlastX-analysis of the sequence from this tag yielded the highest homology to an arylesterase from *Agrobacterium tumefaciens*, with 70% identity. The single clone carrying the corresponding gene was identified (M4aE11) and the full length sequence determined to be as shown below:

```
  1  atgaagacca ttctcgccta tggcgacagc ctgacctatg gggccaaccc gatcccgggc
       m  k  t   i  l  a   y  q  d  s   l  t  y   g  a  n   p  i  p  g

 61  gggccgcggc atgcctatga ggatcgctgg cccacggcgc tggagcaggg gctgggcggc
       g  p  r   h  a  y   e  d  r  w   p  t  a   l  e  q   g  l  g  g

121  aaggcgcggg tgattgccga ggggctgggt ggtcgcacca cggtgcatga cgactggttt
       k  a  r   v  i  a   e  g  l  g   q  r  t  t   v  h   d  d  w  f

181  gcgaatgcgg acaggaacgg tgcgcgggtg ctgccgacgc tgctcgagag ccattcgccg
       a  n  a   d  r  n   g  a  r  v   l  p  t   l  l  e   s  h  s  p

241  ctcgacctga tcgtcatcat gctcggcacc aacgacatca agccgcatca cgggcggacg
```

```
         l  d  l   i  v  i   m  l  q   t  n  d   i  k  p   h  h  g   r  t

  301  gccggcgagg ccgggcgggg catggcgcgg ctggtgcaga tcatccgcgg gcactatgcc
         a  g  e   a  g  r   g  m  a   r  l  v   q  i  i   r  g  h   y  a

  361  ggccgcatgc aggacgagcc gcagatcatc ctcgtgtcgc cgccgccgat catcctcggc
        g  r  m   q  d  e   p  q  i   i  l  v   s  p  p   p  i  i   l  g

  421  gactgggcgg acatgatgga ccatttcggc ccgcacgaag cgatcgccac ctcggtggat
        d  w  a   d  m  m   d  h  f   g  p  h   e  a  i   a  t  s   v  d

  481  ttcgctcgcg agtacaagaa gcgggccgac gagcagaagg tgcatttctt cgacgccggc
        f  a  r   e  y  k   k  r  a   d  e  q   k  v  h   f  f  d   a  g

  541  acggtggcga cgaccagcaa ggccgatggc atccacctcg acccggccaa tacgcgcgcc
        t  v  a   t  t  s   k  a  d   q  i  h   l  d  p   a  n  t   r  a

  601  atcggggcag ggctggtgcc gctggtgaag caggtgctcg gcctgtaa(SEQ ID NO:129)
        i  g  a   g  l  v   p  l  v   k  q  v   l  g  l  -(SEQ ID NO:130)
```

In the above sequence, the conserved structural motifs are shown in bold and underlined. The BlastP-analysis with the deduced full length amino acid sequence identified the same hit with a identity of 48%. The primary structure of this enzyme showed the "GRTT"-motif proving the usefulness of the primers directed towards block 2 for the identification of "GRTT"-esterases. The gene was amplified to introduce unique restriction enzyme recognition sites and the absence of second site mutations was confirmed by sequencing. The gene was ligated to pET26 and was expressed in *E. coli* Rosetta (DE3). The vector map is provided in Figure 5. Expression and control strains were cultivated in LB in the presence of kanamycin (25 μg/ml), chloramphenicol (12.5 μg/ml), and 1% glucose. At an OD_{580} of 1, expression was induced by addition of 100 μM IPTG. Samples were taken at 2, 4, and 20 hours after induction. Cells were separated from the culture supernatant by centrifugation and after resuspending in sample buffer, they wee incubated for 10 minutes at 90°C. An amount of cells representing an OD_{580} of 0.1 was applied to a 4-12% acryl amide gradient gel, which was stained with Coomassie Brilliant Blue R250.

Strong overexpression of the gene was detected already 2 h after induction with 100 µM IPTG, as determined by SDS-PAGE analysis of crude cell extracts from *E. coli* Rosetta (DE3) pET26_M4aE11. The amount of protein representing M4aE11 (calculated size 23.2 kDa) increased further over time.

Esterase activity of crude cell extracts from strains expressing the "GDSL"-type esterase M4aE11 was determined. An amount of cells corresponding to an $O.D._{580} = 2$ were resuspended in 200 µl of 5mM Tris/HCl pH 8.0, and lysed by ultrasonication. Then, 20 µl of each sample were used to determine the esterase activity towards *p*-nitrophenyl butyrate in a total volume of 200 µl. The activity was corrected for the background activity of the control strain. The activity towards *p*-nitrophenylbutyrate reached about 125 nmol/ml x min 20 h after induction.

In addition, SDS-PAGE analysis of the soluble and insoluble fraction of crude cell extracts from *E. coli* Rosetta (DE3) pET26_M4aE11 was conducted. Cells from a culture induced with 100 µM IPTG and harvested 4 h and 20h after induction were lysed by ultrasonication and separated into soluble and insoluble fraction by centrifugation. Sample buffer was added and directly comparable amounts of soluble and insoluble fractions were applied to a 4-12% acryl amide gradient gel, which was stained with Coomassie Brilliant Blue R250. The results of this analysis of the solubility revealed that M4aE11 is partially (estimated 80%) soluble. The screening of the library M091 was completed.

Thus, in total nine different "GDSL"-type esterases were identified in 6 different large insert metagenomic libraries and the esterases/lipases BRAIN's library comprising more than 4.3 Gbp. Eight of these genes were heterologously expressed in *E. coli*. The resulting enzyme samples of seven clones were characterized for the desired perhydrolase activity. Two of the enzymes displayed this activity. Table 3 summarizes the screening, expression and characterization of the metagenomic "GDSL"-type esterases.

Table 3: Expression and Characterization of Metagenomic "GDSL"-Type Esterases

GDSL -type Esterase	Homology[1]	Expression[2] Level	Solubility[3]	Activity[4]	Perhydrolase Activity
S248_M2bB11	12.9%	++	+	136	-
S248_M40cD4	14.8%	+++	++	50	-/+[6]
S248_M44aA5	12.4%	+++	++	75	-/+
S261_M2aA12	36.9%	++	++	72	+[7]
S279_M70aE8	11.9%	+++	+	167	-
S279_M75bA2	5.7%	n.d[5].	n.d.	n.d.	n.d.[5]
M091_M4aE11	33.9%	+++	++	125	n.d.
Est105	4.3%	+++	-	-	n.d.
Est114	7.8%	n.d.	n.d.	13	-

[1] identity to the prototype enzyme from *M. smegmatis* calculated with the dialign algorithm (Morgenstern *et al.*, 1996)

[2] expression level: + moderate overexpression; ++ strong overexpression; +++ very strong overexpression as judged from SDS-PAGE-analysis

[3] as judged by SDS-PAGE-analysis

[4] towards *p*-nitrophenyl butyrate; given as nmol/(ml x min)

[5] not determined

[6] perhydrolysis activity 2x background

[7] perhydrolase activity more than 2x background

Engineering of the Perhydrolase

Based on the structure of the perhydrolase, residues which may alter substrate specificity (*e.g.*, Km, kcat, Vmax, chain length, etc.) and or the multimeric nature of the protein were identified. However, it is not intended that the present invention be limited to any particular residues. Nonetheless, site saturation libraries of residues D10, L12, T13, W14, W16, S54, A55, N94, K97, Y99, P146, W149, F150, I194, F196, are constructed, as well as combinatorial libraries of residues: E51A, Y73A, H81D, T127Q and single mutations of the active site residues D192A, H195A and a site saturation library of the conserved D95. Methods for production of such libraries are known to those skilled in the art and include commercially available kits as the Stratagene Quikchange^{TM} Site-directed mutagenesis kit and/or Quikchange^{TM} Multi-Site-directed mutagenesis kit.

Perhydrolase Activity

The use of enzymes obtained from microorganisms is long-standing. Indeed there are numerous biocatalysts known in the art. For example, U.S. Patent No. 5,240,835 (herein incorporated by reference) provides a description of the transacylase activity of obtained from *C. oxydans* and its production. In addition, U.S. Patent No. 3,823,070 (herein incorporated by reference) provides a description of a *Corynebacterium* that produces certain fatty acids from an n-paraffin. U.S. Patent No. 4,594,324 (herein incorporated by reference) provides a description of a *Methylcoccus capsulatus* that oxidizes alkenes. Additional biocatalysts are known in the art (*See e.g.,* U.S. Patent Nos. 4,008,125 and 4,415,657; both of which are herein incorporated by reference). EP 0 280 232 describes the use of a *C. oxydans* enzyme in a reaction between a diol and an ester of acetic acid to produce monoacetate. Additional references describe the use of a *C. oxydans* enzyme to make chiral hydroxycarboxylic acid from a prochiral diol. Additional details regarding the activity of the *C. oxydans* transacylase as well as the culture of *C. oxydans*, preparation and purification of the enzyme are provided by U.S. Patent No. 5,240,835 (incorporated by reference, as indicated above). Thus, the transesterification capabilities of this enzyme, using mostly acetic acid esters were known. However, the determination that this enzyme could carry out perhydrolysis reaction was quite unexpected. It was even more surprising that these enzymes exhibit very high efficiencies in perhydrolysis reactions. For example, in the presence of tributyrin and water, the enzyme acts to produce butyric acid, while in the presence of tributyrin, water and hydrogen peroxide, the enzyme acts to produce mostly peracetic acid and very little butyric acid. This high perhydrolysis to hydrolysis ratio is a unique property exhibited by the perhydrolase class of enzymes of the present invention and is a unique characteristic that is not exhibited by previously described lipases, cutinases, nor esterases.

The perhydrolase of the present invention is active over a wide pH and temperature range and accepts a wide range of substrates for acyl transfer. Acceptors include water (hydrolysis), hydrogen peroxide (perhydrolysis) and alcohols (classical acyl transfer). For perhydrolysis measurements, enzyme is incubated in a buffer of choice at a specified temperature with a substrate ester in the presence of hydrogen peroxide. Typical substrates used to measure perhydrolysis include esters such as ethyl acetate, triacetin, tributyrin, ethoxylated neodol acetate esters, and others. In addition, the wild type enzyme hydrolyzes nitrophenylesters of short chain acids. The latter are convenient substrates to measure enzyme concentration. Peracid and acetic acid can be measured by the assays described herein. Nitrophenylester hydrolysis is also described.

Although the primary example used during the development of the present invention is the *M. smegmatis* perhydrolase, any perhydrolase obtained from any source which converts the ester into mostly peracids in the presence of hydrogen peroxide finds use in the present invention.

Substrates

In some preferred embodiments of the present invention, esters comprising aliphatic and/or aromatic carboxylic acids and alcohols are utilized with the perhydrolase enzymes of the present invention. In some preferred embodiments, the substrates are selected from one or more of the following: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. In additional embodiments, triacetin, tributyrin, neodol esters, and/or ethoxylated neodol esters serve as acyl donors for peracid formation.

Cleaning and Detergent Formulations

The detergent compositions of the present invention are provided in any suitable form, including for example, as a liquid diluent, in granules, in emulsions, in gels, and pastes. When a solid detergent composition is employed, the detergent is preferably formulated as granules. Preferably, the granules are formulated to additionally contain a protecting agent (*See e.g.*, U.S. Appln. Ser. No. 07/642,669 filed January 17, 1991, incorporated herein by reference). Likewise, in some embodiments, the granules are formulated so as to contain materials to reduce the rate of dissolution of the granule into the wash medium (*See e.g.*, U.S. Patent No. 5,254,283, incorporated herein by reference in its entirety). In addition, the perhydrolase enzymes of the present invention find use in formulations in which substrate and enzyme are present in the same granule. Thus, in some embodiments, the efficacy of the enzyme is increased by the provision of high local concentrations of enzyme and substrate (*See e.g.*, U.S. Patent Application Publication US2003/0191033, herein incorporated by reference).

Many of the protein variants of the present invention are useful in formulating various detergent compositions. A number of known compounds are suitable surfactants useful in compositions comprising the protein mutants of the invention. These include nonionic, anionic, cationic, anionic or zwitterionic detergents (*See e.g.*, U.S. Patent Nos 4,404,128 and 4,261,868). A suitable detergent formulation is that described in U.S. Patent No. 5,204,015 (previously incorporated by reference). Those in the art are familiar with the different formulations which find use as cleaning compositions. As indicated above, in some preferred embodiments, the detergent compositions of the present invention employ a surface active agent (*i.e.*, surfactant) including anionic, non-ionic and ampholytic surfactants well known for their use in detergent compositions. Some surfactants suitable for use in the present invention are described in British Patent Application No. 2 094 826 A, incorporated herein by reference. In some embodiments, mixtures surfactants are used in the present invention.

Suitable anionic surfactants for use in the detergent composition of the present invention include linear or branched alkylbenzene sulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefin sulfonates; alkane sulfonates and the like. Suitable counter ions for anionic surfactants include alkali metal ions such as sodium and potassium; alkaline earth metal ions such as calcium and magnesium; ammonium ion; and alkanolamines having 1 to 3 alkanol groups of carbon number 2 or 3.

Ampholytic surfactants that find use in the present invention include quaternary ammonium salt sulfonates, betaine-type ampholytic surfactants, and the like. Such ampholytic surfactants have both the positive and negative charged groups in the same molecule.

Nonionic surfactants that find use in the present invention generally comprise polyoxyalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like.

In some preferred embodiments, the surfactant or surfactant mixture included in the detergent compositions of the present invention is provided in an amount from about 1 weight percent to about 95 weight percent of the total detergent composition and preferably from about 5 weight percent to about 45 weight percent of the total detergent composition. In various embodiments, numerous other components are included in the compositions of the present invention. Many of these are described below. It is not intended that the present invention be limited to these specific examples. Indeed, it is contemplated that additional compounds will find use in the present invention. The descriptions below merely illustrate some optional components.

Proteins, particularly the perhydrolase of the present invention can be formulated into known powdered and liquid detergents having pH between 3 and 12.0, at levels of about .001 to about 5% (preferably 0.1% to 0.5%) by weight. In some embodiments, these detergent cleaning compositions further include other enzymes such as proteases, amylases, mannanases, peroxidases, oxido reductases, cellulases, lipases, cutinases, pectinases, pectin lyases, xylanases, and/or endoglycosidases, as well as builders and stabilizers.

In addition to typical cleaning compositions, it is readily understood that perhydrolase variants of the present invention find use in any purpose that the native or wild-type enzyme is used. Thus, such variants can be used, for example, in bar and liquid soap applications, dishcare formulations, surface cleaning applications, contact lens cleaning solutions or products, , waste treatment, textile applications, pulp-bleaching, disinfectants, skin care, oral care, hair care, etc. Indeed, it is not intended that any variants of the perhydrolase of the present invention be limited to any particular use. For example, the variant perhydrolases of the present invention may comprise, in addition to decreased allergenicity, enhanced performance in a detergent composition (as compared to the wild-type or unmodified perhydrolase).

The addition of proteins to conventional cleaning compositions does not create any special use limitations. In other words, any temperature and pH suitable for the detergent are also suitable for the present compositions, as long as the pH is within the range in which the enzyme(s) is/are active, and the temperature is below the described protein's denaturing temperature. In addition, proteins of the invention find use in cleaning, bleaching, and disinfecting compositions without detergents, again either alone or in combination with a source of hydrogen peroxide, an ester substrate (*e.g.*, either added or inherent in the system utilized, such as with stains that contain esters, pulp that contains esters etc), other enzymes, surfactants, builders, stabilizers, etc. Indeed it is not intended that the present invention be limited to any particular formulation or application.

Substrates

In some preferred embodiments of the present invention, esters comprising aliphatic and/or aromatic carboxylic acids and alcohols are utilized with the perhydrolase enzymes in the detergent formulations of the present invention. In some preferred embodiments, the substrates are selected from one or more of the following: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, nonanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, and oleic acid. Thus, in some preferred embodiments, detergents comprising at least one perhydrolase, at least one hydrogen peroxide source, and at least one ester acid are provided.

Hydrolases

In addition to the perhydrolase described herein, various hydrolases find use in the present invention, including but not limited to carboxylate ester hydrolase, thioester hydrolase, phosphate monoester hydrolase, and phosphate diester hydrolase which act on ester bonds; a thioether hydrolase which acts on ether bonds; and α-amino-acyl-peptide hydrolase, peptidyl-amino acid hydrolase, acyl-amino acid hydrolase, dipeptide hydrolase, and peptidyl-peptide hydrolase which act on peptide bonds, all these enzymes having high perhydrolysis to hydrolysis ratios (*e.g.*, >1). Preferable among them are carboxylate ester hydrolase, and peptidyl-peptide hydrolase. Suitable hydrolases include: (1) proteases belonging to the peptidyl-peptide hydrolase class (*e.g.*, pepsin, pepsin B, rennin, trypsin, chymotrypsin A, chymotrypsin B, elastase, enterokinase, cathepsin C, papain, chymopapain, ficin, thrombin, fibrinolysin, renin, subtilisin, aspergillopeptidase A, collagenase, clostridiopeptidase B, kallikrein, gastrisin, cathepsin D, bromelin, keratinase, chymotrypsin C, pepsin C, aspergillopeptidase B, urokinase, carboxypeptidase A and B, and aminopeptidase); (2) carboxylate ester hydrolase including carboxyl esterase, lipase, pectin esterase, and chlorophyllase; and (3) enzymes having high perhydrolysis to hydrolysis ratios. Especially effective among them are lipases, as well as esterases that exhibit high perhydrolysis to hydrolysis ratios, as well as protein engineered esterases, cutinases, and lipases, using the primary, secondary, tertiary, and/or quaternary structural features of the perhydrolases of the present invention.

The hydrolase is incorporated into the detergent composition as much as required according to the purpose. It should preferably be incorporated in an amount of 0.0001 to 5 weight percent, and more preferably 0.02 to 3 weight percent,. This enzyme should be used in the form of granules made of crude enzyme alone or in combination with other enzymes and/or components in the detergent composition. Granules of crude enzyme are used in such an amount that the purified enzyme is 0.001 to 50 weight percent in the granules. The granules are used in an amount of 0.002 to 20 and preferably 0.1 to 10 weight percent. In some embodiments, the granules are formulated so as to contain an enzyme protecting agent and a dissolution retardant material (*i.e.*, material that regulates the dissolution of granules during use).

Cationic Surfactants and Long-Chain Fatty Acid Salts

Such cationic surfactants and long-chain fatty acid salts include saturated or fatty acid salts, alkyl or alkenyl ether carboxylic acid salts, a-sulfofatty acid salts or esters, amino acid-type surfactants, phosphate ester surfactants, quaternary ammonium salts including those having 3 to 4 alkyl substituents and up to 1 phenyl substituted alkyl substituents. Suitable cationic surfactants and long-chain fatty acid salts include those disclosed in British Patent Application No. 2 094 826 A, the disclosure of which is incorporated herein by reference. The composition may contain from about 1 to about 20 weight percent of such cationic surfactants and long-chain fatty acid salts.

Builders

In some embodiments of the present invention, the composition contains from about 0 to about 50 weight percent of one or more builder components selected from the group consisting of alkali metal salts and alkanolamine salts of the following compounds: phosphates, phosphonates, phosphonocarboxylates, salts of amino acids, aminopolyacetates high molecular electrolytes, non-dissociating polymers, salts of dicarboxylic acids, and aluminosilicate salts. Examples of suitable divalent sequestering agents are disclosed in British Patent Application No. 2 094 826 A, the disclosure of which is incorporated herein by reference.

In additional embodiments, compositions of the present invention contain from about 1 to about 50 weight percent, preferably from about 5 to about 30 weight percent, based on the composition of one or more alkali metal salts of the following compounds as the alkalis or inorganic electrolytes: silicates, carbonates and sulfates as well as organic alkalis such as triethanolamine, diethanolamine, monoethanolamine and triisopropanolamine.

Anti-Redeposition Agents

In yet additional embodiments of the present invention, the compositions contain from about 0.1 to about 5 weight percent of one or more of the following compounds as antiredeposition agents: polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone and carboxymethylcellulose. In some preferred embodiments, a combination of carboxymethyl-cellulose and/or polyethylene glycol are utilized with the composition of the present invention as useful dirt removing compositions.

Bleaching Agents

The use of the perhydrolases of the present invention in combination with additional bleaching agent(s) such as sodium percarbonate, sodium perborate, sodium sulfate/hydrogen peroxide adduct and sodium chloride/hydrogen peroxide adduct and/or a photo-sensitive bleaching dye such as zinc or aluminum salt of sulfonated phthalocyanine further improves the detergent effects. In additional embodiments, the perhydrolases of the present invention are used in combination with bleach boosters (*e.g.*, TAED and/or NOBS).

Bluing Agents and Fluorescent Dyes

In some embodiments of the present invention, bluing agents and fluorescent dyes are incorporated in the composition. Examples of suitable bluing agents and fluorescent dyes are disclosed in British Patent Application No. 2 094 826 A, the disclosure of which is incorporated herein by reference.

Caking Inhibitors

In some embodiments of the present invention in which the composition is powdered or solid, caking inhibitors are incorporated in the composition. Examples of suitable caking inhibitors include p-toluenesulfonic acid salts, xylenesulfonic acid salts, acetic acid salts, sulfosuccinic acid salts, talc, finely pulverized silica, clay, calcium silicate (*e.g.*, Micro-Cell by Johns Manville Co.), calcium carbonate and magnesium oxide.

Antioxidants

The antioxidants include, for example, tert-butyl-hydroxytoluene, 4,4'-butylidenebis(6-tert-butyl-3-methylphenol), 2,2'-butylidenebis(6-tert-butyl-4-methylphenol), monostyrenated cresol, distyrenated cresol, monostyrenated phenol, distyrenated phenol and 1,1-bis(4-hydroxy-phenyl)cyclohexane.

Solubilizers

In some embodiments, the compositions of the present invention also include solubilizers, including but not limited to lower alcohols (*e.g.*, ethanol, benzenesulfonate salts, and lower alkylbenzenesulfonate salts such as p-toluenesulfonate salts), glycols such as propylene glycol, acetylbenzene-sulfonate salts, acetamides, pyridinedicarboxylic acid amides, benzoate salts and urea.

In some embodiments, the detergent composition of the present invention are used in a broad pH range of from acidic to alkaline pH. In a preferred embodiment, the detergent composition of the present invention is used in mildly acidic, neutral or alkaline detergent wash media having a pH of from above 4 to no more than about 12.

In addition to the ingredients described above, perfumes, buffers, preservatives, dyes and the like also find use with the present invention. These components are provided in concentrations and forms known to those in the art.

In some embodiments, the powdered detergent bases of the present invention are prepared by any known preparation methods including a spray-drying method and a granulation method. The detergent base obtained particularly by the spray-drying method and/or spray-drying granulation method are preferred. The detergent base obtained by the spray-drying method is not restricted with respect to preparation conditions. The detergent base obtained by the spray-drying method is hollow granules which are obtained by spraying an aqueous slurry of heat-resistant ingredients, such as surface active agents and builders, into a hot space. After the spray-drying, perfumes, enzymes, bleaching agents, inorganic alkaline builders may be added. With a highly dense, granular detergent base obtained such as by the spray-drying-granulation method, various ingredients may also be added after the preparation of the base.

When the detergent base is a liquid, it may be either a homogeneous solution or an inhomogeneous dispersion.

The detergent compositions of this invention may be incubated with fabric, for example soiled fabrics, in industrial and household uses at temperatures, reaction times and liquor ratios conventionally employed in these environments. The incubation conditions (*i.e.,* the conditions effective for treating materials with detergent compositions according to the present invention), are readily ascertainable by those of skill in the art. Accordingly, the appropriate conditions effective for treatment with the present detergents correspond to those using similar detergent compositions which include wild-type perhydrolase.

As indicated above, detergents according to the present invention may additionally be formulated as a pre-wash in the appropriate solution at an intermediate pH where sufficient activity exists to provide desired improvements softening, depilling, pilling prevention, surface fiber removal or cleaning. When the detergent composition is a pre-soak (*e.g.,* pre-wash or pre-treatment) composition, either as a liquid, spray, gel or paste composition, the perhydrolase enzyme is generally employed from about 0.00001% to about 5% weight percent based on the total weight of the pre-soak or pre-treatment composition. In such compositions, a surfactant may optionally be employed and when employed, is generally present at a concentration of from about 0.0005 to about 1 weight percent based on the total weight of the pre-soak. The remainder of the composition comprises conventional components used in the pre-soak (*e.g.*, diluent, buffers, other enzymes (proteases), etc.) at their conventional concentrations.

Cleaning Compositions Comprising Perhydrolase

The cleaning compositions of the present invention may be advantageously employed for example, in laundry applications, hard surface cleaning, automatic dishwashing applications, as well as cosmetic applications such as dentures, teeth, hair and skin. However, due to the unique advantages of increased effectiveness in lower temperature solutions and the superior color-safety profile, the enzymes of the present invention are ideally suited for laundry applications such as the bleaching of fabrics. Furthermore, the enzymes of the present invention find use in both granular and liquid compositions.

The enzymes of the present invention also find use in cleaning additive products. Cleaning additive products including the enzymes of the present invention are ideally suited for inclusion in wash processes where additional bleaching effectiveness is desired. Such instances include, but are not limited to low temperature solution cleaning applications. The additive product may be, in its simplest form, one or more of the enzymes of the present invention. Such additive may be packaged in dosage form for addition to a cleaning process where a source of peroxygen is employed and increased bleaching effectiveness is desired. Such single dosage form may comprise a pill, tablet, gelcap or other single dosage unit such as pre-measured powders or liquids. A filler or carrier material may be included to increase the volume of such composition. Suitable filler or carrier materials include, but are not limited to, various salts of sulfate, carbonate and silicate as well as talc, clay and the like. Filler or carrier materials for liquid compositions may be water or low molecular weight primary and secondary alcohols including polyols and diols. Examples of such alcohols include, but are not limited to; methanol, ethanol, propanol and isopropanol. The compositions may contain from about 5% to about 90% of such materials. Acidic fillers can be used to reduce pH. Alternatively, the cleaning additive may include activated peroxygen source defined below or the adjunct ingredients as defined below.

The cleaning compositions and cleaning additives of the present invention require an effective amount of the enzymes provided by the present invention. The required level of enzyme may be achieved by the addition of one or more species of the *M. smegmatis* perhydrolase, variants, homologues, and/or other enzymes or enzyme fragments having the activity of the enzymes of the present invention. Typically, the cleaning compositions of the present invention comprise at least 0.0001 weight percent, from about 0.0001 to about 1, from about 0.001 to about 0.5, or even from about 0.01 to about 0.1 weight percent of at least one enzyme of the present invention.

In some embodiments, the cleaning compositions of the present invention comprise a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, said peroxygen source being selected from the group consisting of:

(i) from about 0.01 to about 50, from about 0.1 to about 20, or even from about 1 to 10 weight percent of a per-salt, an organic peroxyacid, urea hydrogen peroxide and mixtures thereof;

(ii) from about 0.01 to about 50, from about 0.1 to about 20, or even from about 1 to 10 weight percent of a carbohydrate and from about 0.0001 to about 1, from about 0.001 to about 0.5, from about 0.01 to about 0.1 weight percent carbohydrate oxidase; and

(iii) mixtures thereof.

Suitable per-salts include those selected from the group consisting of alkalimetal perborate, alkalimetal percarbonate, alkalimetal perphosphates, alkalimetal persulphates and mixtures thereof.

The carbohydrate may be selected from the group consisting of mono-carbohydrates, di-carbohydrates, tri-carbohydrates, oligo-carbohydrates and mixtures thereof. Suitable carbohydrates include carbohydrates selected from the group consisting of D-arabinose, L-arabinose, D-Cellobiose, 2-Deoxy-D-galactose, 2-Deoxy-D-ribose, D-Fructose, L-Fucose, D-Galactose, D-glucose, D-glycero-D-gulo-heptose, D-lactose, D-Lyxose, L-Lyxose, D-Maltose, D-Mannose, Melezitose, L-Melibiose, Palatinose, D-Raffinose, L-Rhamnose, D-Ribose, L-Sorbose, Stachyose, Sucrose, D-Trehalose, D-Xylose, L-Xylose and mixtures thereof.

Suitable carbohydrate oxidases include carbohydrate oxidases selected from the group consisting of aldose oxidase (IUPAC classification EC1.1.3.9), galactose oxidase (IUPAC classification EC1.1.3.9), cellobiose oxidase (IUPAC classification EC1.1.3.25), pyranose oxidase (IUPAC classification EC1.1.3.10), sorbose oxidase (IUPAC classification EC1.1.3.11) and/or hexose oxidase (IUPAC classification EC1.1.3.5), Glucose oxidase (IUPAC classification EC1.1.3.4) and mixtures thereof.

In some preferred embodiments, the cleaning compositions of the present invention also include from about 0.01 to about 99.9, from about 0.01 to about 50, from about 0.1 to 20, or even from about 1 to about 15 weight percent a molecule comprising an ester moiety. Suitable molecules comprising an ester moiety may have the formula:

$$R^1O_x\,[(R^2)_m\,(R^3)_n]_p$$

wherein R^1 is a moiety selected from the group consisting of H or a substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl; in one aspect of the present invention, R^1 may comprise from 1 to 50,000 carbon atoms, from 1 to 10,000 carbon atoms, or even from 2 to 100 carbon atoms;

each R^2 is an alkoxylate moiety, in one aspect of the present invention, each R^2 is independently an ethoxylate, propoxylate or butoxylate moiety;

R^3 is an ester-forming moiety having the formula:

R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl, in one aspect of the present invention, R^4 may be substituted or unsubstituted alkyl, alkenyl, alkynyl, moiety comprising from 1 to 22 carbon atoms, an aryl, alkylaryl, alkylheteroaryl, or heteroaryl moiety comprising from 4 to 22 carbon atoms or R^4 may be a substituted or unsubstituted C_1-C_{22} alkyl moiety or R^4 may be a substituted or unsubstituted C_1-C_{12} alkyl moiety;

x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1

p is an integer that is equal to or less than x

m is an integer from 0 to 50, an integer from 0 to 18, or an integer from 0 to 12, and n is at least 1.

In one aspect of the present invention, the molecule comprising an ester moiety is an alkyl ethoxylate or propoxylate having the formula $R^1O_x[(R^2)_m(R^3)_n]_p$ wherein:

R^1 is an C_2-C_{32} substituted or unsubstituted alkyl or heteroalkyl moiety;
each R^2 is independently an ethoxylate or propoxylate moiety;
R^3 is an ester-forming moiety having the formula:
R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl, in one aspect of the present invention, R^4 may be a substituted or unsubstituted alkyl, alkenyl, or alkynyl moiety comprising from 1 to 22 carbon atoms, a substituted or unsubstituted aryl, alkylaryl, alkylheteroaryl, or heteroaryl moiety comprising from 4 to 22 carbon atoms or R^4 may be a substituted or unsubstituted C_1-C_{22} alkyl moiety or R^4 may be a substituted or unsubstituted C_1-C_{12} alkyl moiety;
x is an integer that is equal to or less than the number of carbons in R^1
p is an integer that is equal to or less than x
m is an integer from 1 to 12, and
n is at least 1.

In one aspect of the present invention, the molecule comprising the ester moiety has the formula:

$$R^1O_x[(R^2)_m(R^3)_n]_p$$

wherein R^1 is H or a moiety that comprises a primary, secondary, tertiary or quaternary amine moiety, said R^1 moiety that comprises an amine moiety being selected from the group consisting of a substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl; in one aspect of Applicants' invention R^1 may comprise from 1 to 50,000 carbon atoms, from 1 to 10,000 carbon atoms, or even from 2 to 100 carbon atoms;

each R^2 is an alkoxylate moiety, in one aspect of the present invention each R^2 is independently an ethoxylate, propoxylate or butoxylate moiety;

R^3 is an ester-forming moiety having the formula:

R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl, in one aspect of the present invention, R^4 may be a substituted or unsubstituted alkyl, alkenyl, or alkynyl moiety comprising from 1 to 22 carbon atoms, a substituted or unsubstituted aryl, alkylaryl, alkylheteroaryl, or heteroaryl moiety comprising from 4 to 22 carbon atoms or R^4 may be a substituted or unsubstituted C_1-C_{22} alkyl moiety or R^4 may be a substituted or unsubstituted C_1-C_{12} alkyl moiety;

x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1

p is an integer that is equal to or less than x

m is an integer from 0 to 12 or even 1 to 12, and

n is at least 1.

In any of the aforementioned aspects of the present invention, the molecule comprising an ester moiety may have a weight average molecular weight of less than 600,000 Daltons, less than 300,000 Daltons, less than 100,000 Daltons or even less than 60,000 Daltons.

Suitable molecules that comprise an ester moiety include polycarbohydrates that comprise an ester moiety.

The cleaning compositions provided herein will typically be formulated such that, during use in aqueous cleaning operations, the wash water will have a pH of from about 5.0 to about 11.5, or even from about 7.5 to about 10.5. Liquid product formulations are typically formulated to have a pH from about 3.0 and about 9.0. Granular laundry products are typically formulated to have a pH from about 9 to about 11. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When the enzyme(s) of the present invention is/are employed in a granular composition or liquid, it may be desirable for the enzyme(s) to be in the form of an encapsulated particle to protect such enzyme from other components of the granular composition during storage. In addition, encapsulation is also a means of controlling the availability of the enzyme(s) during the cleaning process and may enhance performance of the enzyme(s). In this regard, the enzyme(s) may be encapsulated with any encapsulating material known in the art.

The encapsulating material typically encapsulates at least part of the enzyme(s). Typically, the encapsulating material is water-soluble and/or water-dispersible. The encapsulating material may have a glass transition temperature (Tg) of 0°C or higher. Glass transition temperature is described in more detail in WO 97/11151, especially from page 6, line 25 to page 7, line 2.

The encapsulating material may be selected from the group consisting of carbohydrates, natural or synthetic gums, chitin and chitosan, cellulose and cellulose derivatives, silicates, phosphates, borates, polyvinyl alcohol, polyethylene glycol, paraffin waxes and combinations thereof. When the encapsulating material is a carbohydrate, it may be typically selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, and combinations thereof. Typically, the encapsulating material is a starch. Suitable starches are described in EP 0 922 499; US 4,977,252; US 5,354,559 and US 5,935,826.

The encapsulating material may be a microsphere made from plastic such as thermoplastics, acrylonitrile, methacrylonitrile, polyacrylonitrile, polymethacrylonitrile and mixtures thereof; commercially available microspheres that can be used are those supplied by Expancel of Stockviksverken, Sweden under the trademark EXPANCEL®, and those supplied by PQ Corp. of Valley Forge, Pennsylvania U.S.A. under the tradename PM 6545, PM 6550, PM 7220, PM 7228, EXTENDOSPHERES®, LUXSIL®, Q-CEL® and SPHERICEL®.

Processes of Making and Using the Cleaning Compositions of the Present Invention

The cleaning compositions of the present invention can be formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. 5,879,584; U.S. 5,691,297; U.S. 5,574,005; U.S. 5,569,645; U.S. 5,565,422 Del Greco et al.; U.S. 5,516,448; U.S. 5,489,392; and U.S. 5,486,303; all of which are incorporated herein by reference.

Adjunct Materials in Addition to the Enzymes of the Present Invention, Hydrogen Peroxide, and/or Hydrogen Peroxide Source and Material Comprising an Ester Moiety

While not essential for the purposes of the present invention, the non-limiting list of adjuncts illustrated hereinafter are suitable for use in the instant cleaning compositions and may be desirably incorporated in certain embodiments of the invention, for example to assist or enhance cleaning performance, for treatment of the substrate to be cleaned, or to modify the aesthetics of the cleaning composition as is the case with perfumes, colorants, dyes or the like. It is understood that such adjuncts are in addition to the enzymes of the present invention, hydrogen peroxide and/or hydrogen peroxide source and material comprising an ester moiety. The precise nature of these additional components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the cleaning operation for which it is to be used. Suitable adjunct materials include, but are not limited to, surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, additional enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids and/or pigments. In addition to the disclosure below, suitable examples of such other adjuncts and levels of use are found in U.S. Patent Nos. 5,576,282, 6,306,812, and 6,326,348, herein incorporated by reference. The aforementioned adjunct ingredients may constitute the balance of the cleaning compositions of the present invention.

Surfactants - The cleaning compositions according to the present invention may comprise a surfactant or surfactant system wherein the surfactant can be selected from nonionic surfactants, anionic surfactants, cationic surfactants, ampholytic surfactants, zwitterionic surfactants, semi-polar nonionic surfactants and mixtures thereof.

The surfactant is typically present at a level of from about 0.1% to about 60%, from about 1% to about 50% or even from about 5% to about 40% by weight of the subject cleaning composition.

Builders - The cleaning compositions of the present invention may comprise one or more detergent builders or builder systems. When a builder is used, the subject cleaning composition will typically comprise at least about 1%, from about 3% to about 60% or even from about 5% to about 40% builder by weight of the subject cleaning composition.

Builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicate builders polycarboxylate compounds. ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic acid and nitrilotriacetic acid, as well as polycarboxylates such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof.

Chelating Agents - The cleaning compositions herein may contain a chelating agent, Suitable chelating agents include copper, iron and/or manganese chelating agents and mixtures thereof.

When a chelating agent is used, the cleaning composition may comprise from about 0.1% to about 15% or even from about 3.0% to about 10% chelating agent by weight of the subject cleaning composition.

Deposition Aid - The cleaning compositions herein may contain a deposition aid. Suitable deposition aids include, polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as Kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

Dye Transfer Inhibiting Agents - The cleaning compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof.

When present in a subject cleaning composition, the dye transfer inhibiting agents may be present at levels from about 0.0001% to about 10%, from about 0.01% to about 5% or even from about 0.1% to about 3% by weight of the cleaning composition.

Dispersants - The cleaning compositions of the present invention can also contain dispersants. Suitable water-soluble organic materials include the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Enzymes - The cleaning compositions can comprise one or more detergent enzymes which provide cleaning performance and/or fabric care benefits. Examples of suitable enzymes include, but are not limited to, hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and amylases, or mixtures thereof. A typical combination is cocktail of conventional applicable enzymes like protease, lipase, cutinase and/or cellulase in conjunction with amylase.

Enzyme Stabilizers - Enzymes for use in detergents can be stabilized by various techniques. The enzymes employed herein can be stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions that provide such ions to the enzymes.

Catalytic Metal Complexes - The cleaning compositions of the present invention may include catalytic metal complexes. One type of metal-containing bleach catalyst is a catalyst system comprising a transition metal cation of defined bleach catalytic activity, such as copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations, an auxiliary metal cation having little or no bleach catalytic activity, such as zinc or aluminum cations, and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof. Such catalysts are disclosed in U.S. 4,430,243.

If desired, the compositions herein can be catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art and include, for example, the manganese-based catalysts disclosed in U.S. 5,576,282.

Cobalt bleach catalysts useful herein are known, and are described, for example, in U.S. 5,597,936; and U.S. 5,595,967. Such cobalt catalysts are readily prepared by known procedures, such as taught for example in U.S. 5,597,936, and U.S. 5,595,967.

Compositions herein may also suitably include a transition metal complex of a macropolycyclic rigid ligand - abreviated as "MRL". As a practical matter, and not by way of limitation, the compositions and cleaning processes herein can be adjusted to provide on the order of at least one part per hundred million of the active MRL species in the aqueous washing medium, and will preferably provide from about 0.005 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the MRL in the wash liquor.

Preferred transition-metals in the instant transition-metal bleach catalyst include manganese, iron and chromium. Preferred MRL's herein are a special type of ultra-rigid ligand that is cross-bridged such as 5,12-diethyl-1,5,8,12-tetraazabicyclo[6.6.2] hexadecane.

Suitable transition metal MRLs are readily prepared by known procedures, such as taught for example in WO 00/332601, and U.S. 6,225,464.

Method of Use

The cleaning compositions disclosed herein of can be used to clean a situs *inter alia* a surface or fabric. Typically at least a portion of the situs is contacted with an embodiment of Applicants' cleaning composition, in neat form or diluted in a wash liquor, and then the situs is optionally washed and/or rinsed. For purposes of the present invention, washing includes but is not limited to, scrubbing, and mechanical agitation. The fabric may comprise most any fabric capable of being laundered in normal consumer use conditions. The disclosed cleaning compositions are typically employed at concentrations of from about 500 ppm to about 15,000 ppm in solution. When the wash solvent is water, the water temperature typically ranges from about 5 °C to about 90 °C and, when the situs comprises a fabric, the water to fabric mass ratio is typically from about 1:1 to about 30:1.

EXPERIMENTAL

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: °C (degrees Centigrade); rpm (revolutions per minute); H_2O (water); HCl (hydrochloric acid); aa (amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); gm (grams); µg and ug (micrograms); mg (milligrams); ng (nanograms); µl and ul (microliters); ml (milliliters); mm (millimeters); nm (nanometers); µm and um (micrometer); M (molar); mM (millimolar); µM and uM (micromolar); U (units); V (volts); MW (molecular weight); sec (seconds); min(s) (minute/minutes); hr(s) (hour/hours); $MgCl_2$ (magnesium chloride); NaCl (sodium chloride); OD_{280} (optical density at 280 nm); OD_{600} (optical density at 600 nm); PAGE (polyacrylamide gel electrophoresis); EtOH (ethanol); PBS (phosphate buffered saline [150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2]); SDS (sodium dodecyl sulfate); Tris (tris(hydroxymethyl)aminomethane); TAED (N,N,N'N'-tetraacetylethylenediamine); w/v (weight to volume); v/v (volume to volume); Per (perhydrolase); *per* (perhydrolase gene); Ms (*M. smegmatis*); MS (mass spectroscopy); BRAIN (BRAIN Biotechnology Research and Information Network, AG, Zwingenberg, Germany); TIGR (The Institute for Genomic Research, Rockville, MD); AATCC (American Association of Textile and Coloring Chemists); WFK (wfk Testgewebe GmbH, Bruggen-Bracht, Germany); Amersham (Amersham Life Science, Inc. Arlington Heights, IL); ICN (ICN Pharmaceuticals, Inc., Costa Mesa, CA); Pierce (Pierce Biotechnology, Rockford, IL); Amicon (Amicon, Inc., Beverly, MA); ATCC (American Type Culture Collection, Manassas, VA); Amersham (Amersham Biosciences, Inc., Piscataway, NJ); Becton Dickinson (Becton Dickinson Labware, Lincoln Park, NJ); BioRad (BioRad, Richmond, CA); Clontech (CLONTECH Laboratories, Palo Alto, CA); Difco (Difco Laboratories, Detroit, MI); GIBCO BRL or Gibco BRL (Life Technologies, Inc., Gaithersburg, MD); Novagen (Novagen, Inc., Madison, WI); Qiagen (Qiagen, Inc., Valencia, CA); Invitrogen (Invitrogen Corp., Carlsbad, CA); Genaissance (Genaissance Pharmaceuticals, Inc., New Haven, CT); DNA 2.0 (DNA 2.0, Menlo Park, CA); MIDI

(MIDI Labs, Newark, DE) InvivoGen (InvivoGen, San Diego, CA); Sigma (Sigma Chemical Co., St. Louis, MO); Sorvall (Sorvall Instruments, a subsidiary of DuPont Co., Biotechnology Systems, Wilmington, DE); Stratagene (Stratagene Cloning Systems, La Jolla, CA); Roche (Hoffmann La Roche, Inc., Nutley, NJ); Agilent (Agilent Technologies, Palo Alto, CA); Minolta (Konica Minolta, Ramsey, NJ); and Zeiss (Carl Zeiss, Inc., Thornwood, NY).

In the following Examples, various media were used. "TS" medium (per liter) was prepared using Tryptone (16 g) (Difco), Soytone (4 g) (Difco), Casein hydrolysate (20 g) (Sigma), K_2HPO_4 (10 g), and d H_2O (to 1 L). The medium was sterilized by autoclaving. Then, sterile glucose was added to 1.5% final concentration. Streptomyces Production Medium (per liter) was prepared using citric acid(H_2O) (2.4 g), Biospringer yeast extract (6 g), $(NH_4)2SO_4$ (2.4 g), $MgSO_4 \cdot 7\ H_2O$ (2.4 g), Mazu DF204 (5 ml), trace elements (5 ml). The pH was adjusted to 6.9 with NaOH. The medium was then autoclaved to sterilize. After sterilization, $CaCl_2 \cdot 2\ H_2O$ (2 mls of 100 mg/ml solution), KH_2PO_4 (200 ml of a 13% (w/v) solution at pH6.9), and 20 mls of a 50% glucose solution were added to the medium.

In these experiments, a spectrophotometer was used to measure the absorbance of the products formed after the completion of the reactions. A reflectometer was used to measure the reflectance of the swatches. Unless otherwise indicated, protein concentrations were estimated by Coomassie Plus (Pierce), using BSA as the standard.

EXAMPLE 1

Enzyme Analysis

In this Example, methods to assess enzyme purity and activity used in the subsequent Examples and throughout the present Specification are described.

Enzyme Activity Assay (pNB Assay)

This activity was measured by hydrolysis of *p*-nitrophenylbutyrate. The reaction mixture was prepared by adding 10 ul of 100 mM *p*-nitrophenylbutyrate in dimethylsulfoxide to 990 ml of 100 mM Tris-HCl buffer, pH 8.0 containing 0.1 % triton X-100. The background rate of hydrolysis was measured before the addition of enzyme at 410 nm. The reaction was initiated by the addition of 10 ul of enzyme to 990 ml of the reaction and the change of absorbance at 410 nm was measured at room temperate (~23°C). The background corrected results are reported as δA_{410}/min/ml or δA_{410}/min/mg protein.

Transesterification

Transesterification was measured by GC separation of products in buffered aqueous reactions. Reactions to measure ethyl acetate transesterification with propanol contained in 1 ml of 50 mM KPO4, pH 7.0; 200 mM ethyl acetate, 200 mM 1-propanol, and enzyme. Reactions to measure ethyl acetate transesterification with neopentyl glycol (NPG) contained in 1 ml of 50 mM KPO4, pH 7.0; 303 mM ethyl acetate, 100 mM NPG, and enzyme. The reactions were incubated at the indicated temperatures and for the indicated times. Separations were preformed using a 30M FFAP column (Phenomenex). The inlet split ratio was approximately 1:25, the injector was 250°C, head pressure of 10 psi He, and detection was by FID at 250°C. The chromatography program was 40°C initial for 4 min, followed by a gradient of 15°C/min to 180°C. Components eluted in the following order and were not quantified; ethyl acetate, ethyl alcohol, propyl acetate, propyl alcohol, acetic acid, NPG diacetate, NPG monoacetate, and NPG.

Perhydrolase Used in Crystallography Studies

This perhydrolase preparation was used for crystallography studies. In addition, unlabelled protein was grown and purified in similar manner. A 500 ml preculture of *E. coli* BL21(DE3)/pLysS/pMSATNco1-1 was grown in a baffled 2.8 L Fernbach flask on LB containing 100 ug/ml carbenicillin. After overnight culture at 37°C and 200 rpm on a rotary shaker, the cells were harvested by centrifugation and resuspended in M9 medium containing: glucose, 2 g/L; Na_2HPO_4, 6 g/L; KH_2PO_4, 3 g/L; NH_4Cl, 1 g/L; NaCl, 0.5 g/L; thiamine, 5 mg/L; $MgSO_4$, 2 mM; $CaCl_2$, 100 uM; Citric acid•H_2O, 40 mg/L; $MnSO_4 \bullet H_2O$, 30 mg/L; NaCl, 10 mg/L; $FeSO_4 \bullet 7H_2O$, 1 mg/L; $CoCl_2 \bullet 6H_2O$, 1 mg/L; $ZnSO_4 \bullet 7H_2O$, 1 mg/L; $CuSO_4 \bullet 5H_2O$, 100 ug/L; $H_3BO_3 \bullet 5H_2O$, 100 ug/L; and $NaMoO_4 \bullet 2H_2O$, 100 ug/L; and supplemented with carbenicillin, 100 mg/L. The resuspended cells were used to inoculate six Fernbach flasks containing 500 ml each of M9 medium supplemented with carbenicillin (100 mg/L). The cultures were incubated at 20°C and 200 rpm on a rotary shaker until the OD_{600} reached about 0.7 at which time 100 mg/L of lysine, threonine, and phenylalanine and 50 mg/L of leucine, isoleucine, valine, and selenomethionine were added. After further incubation for 30 min, IPTG was added to a final concentration of 50 uM. The cultures were then incubated overnight (~15hr) and harvested by centrifugation. The cell pellet was washed 2 times with 50 mM KPO_4 buffer, pH 6.8. The yield was 28.5 gm wet weight of cells to which was added 114 ml of 100 mM KPO_4 buffer, pH 8.2 and 5 mg of DNase. This mixture was frozen at -80°C and thawed 2 times.

The thawed cell suspension was lysed by disruption in a French pressure cell at 20K psi. The unbroken cells and cell membrane material were sedimented by centrifugation at 100K times g for 1 hour. The supernatant crude extract, 128 ml (CE) was then placed in a 600 ml beaker and stirred for 10 minutes in a 55°C water bath to precipitate unstable proteins. After 10 min the beaker was stirred in ice water for 1 min followed by centrifugation at 15K times g for 15 min. The supernatant from this procedure, HT, contained 118 ml. The HT extract was then made 20% saturating in $(NH_4)_2SO_4$ by the slow addition of 12.7 g of $(NH_4)_2SO_4$. This was loaded on to a 10 cm

X 11.6 cm Fast Flow Phenyl Sepharose (Pharmacia) column equilibrated in100 mM KPO_4 buffer, pH 6.8, containing 20% saturation (109 g/L) $(NH_4)_2SO_4$. After loading the extract the column was washed with 1700 ml of starting buffer and eluted with a two step gradient. The first step was a linear 1900 ml gradient from start buffer to the same buffer without $(NH_4)_2SO_4$, the second was a 500 ml elution with 100 mM KPO_4, pH 6.8 containing 5% EtOH. Active fractions, 241 ml, were pooled, diluted 100 % with water and loaded onto a 1.6 mm X 16 mm Poros HQ strong anion exchange column equilibrated in 100 mM Tris-HCl, pH 7.6. After loading the extract, the column was washed with 5 column volumes of starting buffer. The protein was eluted with a 15 column volume gradient from start buffer to start buffer containing 175 mM KCl. The active fractions were pooled and concentrated using a Centriprep 30 (Millipore) to 740 µl. Figure 6 provides a purification table showing the enzyme activity of the enzyme of the present invention through various steps in the purification process.

The present application must be used to determine the respective values of the parameters of the present invention.

Unless otherwise noted, all component or composition levels are in reference to the active level of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources.

Enzyme components weights provided herein are based on total active protein. All percentages and ratios were calculated by weight unless otherwise indicated. All percentages and ratios were calculated based on the total composition unless otherwise indicated.

EXAMPLE 2

Determination of Ratio Between Peracid and Acid Formation

In this Example, methods for determining the ratio of perhydrolysis to hydrolysis are described. In particular, this Example provides methods for determining the ratio between peracid formation (*i.e.*, perhydrolysis) and acid formation (*i.e.*, hydrolysis) resulting from enzyme activity on an ester substrate in the presence of peroxide in an aqueous system.

A. Determination of Perhydrolysis to Hydrolysis Ratio

Preparation of Substrate

The substrates were prepared as described herein. Ethyl acetate (EtOAc) and other water soluble esters were diluted in a desired buffer to a concentration of 10 mM of ester. Tributyrin and other water insoluble substrates were prepared by making substrate swatches. Polyester swatches were cut from non-dyed polyester fabric (Polycotton, PCW 22) using a 5/8 inch punch and placed in a 24-well microtiter plate (Costar, Cell Culture Plate). The insoluble ester was diluted to 1.03 M in hexane. Then, 10 µL of the insoluble ester solution were then adsorbed onto the polyester swatch.

Determination of Hydrolysis (GC Assay)

The hydrolytic assay described below was used to determine the amount of substrate hydrolysis. In this assay, the assay solution was comprised of 50 mM potassium phosphate pH 7.5, 10 mM ester substrate, 29 mM hydrogen peroxide, and 20 mM potassium chloride in a total volume of 0.99ml and an amount of enzyme that would generate 20 nmoles of acetic acid per minute at 25°C.

For measuring water insoluble ester hydrolysis, the reaction mixture was added to the insoluble ester fabric swatch. The swatch was prepared as described above ("Preparation of Substrate"). All the other conditions for the assay were the same except for exclusion of other ester substrates.

Hydrolytic activity was measured by monitoring the increase of acids generated by the enzyme from acyl donor substrates using gas chromatography coupled with flame ionization detection. The assay was conducted by first pipetting 50 μL of assay solution containing all the components except the enzyme into 200 mL of methanol (HPLC grade) to determine the amount of acid in the assay solution at time 0. Then, 10 μL of enzyme were added to the assay solution to a desired final concentration which produced approximately 20 nanomoles of acid per minute. A timer was started and 50 μL aliquots were taken from the assay solution and added to 200 μL of methanol at various times, typically 2, 5, 10, 15, 25, 40, and 60 minutes, after addition of the enzyme.

These methanol-quenched samples were then injected into a gas chromatograph coupled with a flame ionization detector (Agilent 6890N) and analyzed for hydrolytic components, acetic, and butyric acids. Gas chromatography was conducted using a nitroterephthalic acid modified polyethylene glycol column (Zebron FFAP; with dimensions: 30 m long, 250 um diameter, 250 nm film thickness). A 3 μL aliquot of sample was applied to the column by a splitless injection under constant a helium flow of 1.0 mL/minute. The inlet was maintained at a temperature of 250°C and was purged of any remaining sample components after 2 minutes. When analyzing acetic acid, the temperature of the column was maintained at 75°C for 1 minute after injection, increased 25°C/minute to 100°C, then increased 15°C/minute to 200°C.

When analyzing butyric acid, the temperature of the column was controlled as described above, except the temperature was additionally increased 25°C/minute to 225°C and held at 225°C for 1 minute. The flame ionization detector was maintained throughout the chromatography at 250°C and under constant hydrogen flow of 25 mL/minute, air flow of 200 mL/minute, and a combined column and makeup helium flow of 30 mL/minute. The amount of hydrolyzed acid in the sample was then determined by integrating the acid peak in the chromatogram for total ion counts and calculating the acid from the ion count using a standard curve generated under the above conditions for acetic and butyric acids at varying concentrations in the assay solution (without enzyme).

Determination of Perhydrolysis (OPD Assay)

The perhydrolytic activity assay described below was used to determine the amount of peracid formed in the reaction. In these assays, the solution comprised 50 mM potassium phosphate pH 7.5, 10 mM ester substrate, 29 mM hydrogen peroxide, 20 mM potassium chloride, and 10 mM O-phenylenediamine.

When using water insoluble ester as the acyl donor, an ester adsorbed fabric swatch was used as the substrate, prepared as described above ("Preparation of Substrate").

Perhydrolytic activity was measured by monitoring the absorbance increase at 458 nm of oxidized O-phenylenediamine (OPD) by peracid generated with the enzyme. The perhydrolytic activity assay solution was prepared in the same manner as the hydrolytic activity assay solution, except that OPD was added to the assay solution to a final concentration of 10mM. The OPD solution was prepared immediately before conducting the assay by dissolving 72mg OPD (Sigma-Aldrich, dihydrochloride) in 19.94 mL of the same buffer and the pH was adjusted by slowly adding 60 µL of 13.5 M potassium hydroxide. The pH was measured and if needed, small quantities of potassium hydroxide were added to return the pH to the original pH of the buffer. Then, 495 µL of this OPD solution were added with the other assay components to a final assay volume of 0.990 mL. An assay quenching solution was also prepared by dissolving 36mg OPD in 20 mL 100 mM citric acid and 70% ethanol.

The assay was typically conducted at 25°C. The assay was started by pipetting 100 µL of assay solution before the addition of the enzyme into 200 µL of quenching solution to determine the amount of perhydrolytic components and background absorbance in the assay solution at time 0. Then, 10 µL of enzyme were added to the assay solution to a desired final concentration which produced approximately 10 nanomoles of peracid per minute. A timer was started and 100 μL aliquots were taken from the assay solution and added to 200 μL of quenching solution at various times, typically 2, 5, 10, 15, 25, 40, and 60 minutes, after adding the enzyme. The quenched assay solutions were incubated for 30 minutes to allow any remaining peracid to oxidize the OPD. Then, 100 μL of each quenched assay solution was transferred to a 96-well microtiter plate (Costar) and the absorbance of the solution was measured at 458 nm by a spectrophotometric plate reader (Molecular Devices, SpectraMAX 250). The amount of peracid in each quenched sample was calculated using a standard curve generated under the above conditions with peracetic acid at varying concentrations in the assay solution (without enzyme).

Perhydrolysis /Hydrolysis ratio:

Perhydrolysis/ Hydrolysis ratio= Perhydrolysis measured in the Perhydrolysis assay/(Total acid detected in the hydrolysis assay-Perhydrolysis measured in the perhydrolysis assay)

The results of these experiments are provided in Figures 7, 10 and Figure 11. Figure 7 provides a graph which shows the ratio of perbutyric acid to butyric acid generated by various enzymes from 10 mM tributyrin and 29 mM hydrogen peroxide in 40 minutes. Figure 10 shows the ratio of perbutyric acid to butyric acid generated by various enzymes from 10 mM tributyrin and 29 mM hydrogen peroxide in 4, 10, and 30 minutes. Figure 11 shows the ratio of peracetic acid to acetic acid generated by various enzymes from 10 mM triacetin and 29 mM hydrogen peroxide in 4 and 10 minutes. The results obtained in these experiments indicated that *M. smegmatis* perhydrolase homologues exhibited a ratio above 1 in the OPD/GC assays described above, while other classes of enzymes exhibited ratios significantly below 1.

Table 2-1provides data showing the perhydrolysis activity of various homologues described herein on triacetin, as compared to the wild-type *M. smegmatis* perhydrolase. The results provided in Table 2-2 indicate that the perhydrolase has activity over a broad range of substrates. In addition to the results provided in these Tables, Figures 8 and 9 provide data showing that the perhydrolase of the present invention has broad pH and temperature range activities.

Table 2-1. Perhydrolysis Activity of Perhydrolase Homologues on Triacetin as Compared to *M. smegmatis* perhydrolase

Experiment	Protein	Perhydrolysis Ratio (homolog to perhydrolase)
A.		
	pET26_Mlo	0.6
	pET26b_Mbo	0.87
	pET26_SmeII	2.1
	pET26b_Stm	0.17
	pLO_SmeI	0.7
	Perhydrolase	1.0000
	Blank	0.0660
B.	pET26_S261_M2aA12	1.5
	Perhydrolase	1
	Blank	0.3
C.	pet26 M40cD4	0.14
	pet26 M44aA5	0.16
	Perhydrolase	1
	Blank	0.01

Table 2-2. Peracid Production by 1 ppm Wild-Type Perhydrolase with 29 mM H2O2 and Various Esters

nmol Peracetic Acid / min

Ester	10mM of Ester with 0.5% Neodol	10mM of Ester	10mM of Ester on Polycotton Swatch
Ethyl Acetate		5.00	
Butyl Acetate	8.06	8.72	
Hexyl Acetate	7.96	5.86	
Octyl Acetate	8.03	0.48	
Ethyl Propionate	0.90	1.43	
Butyl Propionate	2.47	3.39	
Hexyl Propionate	4.00	2.66	
Isoamyl Acetate	7.83		17.69
Citronellyl Acetate	7.25		4.27
Citronellyl Propionate	2.85		3.21
Dodecyl Acetate	3.95		0.19
Neodol 23-3 Acetate	2.25		8.77
Neodol 23-6.5 Acetate	2.73		10.12
Neodol 23-9 Acetate	2.97		10.20
Ethylene Glycol Diacetate	13.30		
Propylene Glycol Diacetate	13.17		
Triacetin	11.91		
Tributyrin	0.66		2.70
Ethyl Methoxyacetate	0.49		
Linalyl Acetate	0.30		
Ethyl Butyrate	0.31		
Ethyl Isobutyrate	0.10		
Ethyl 2-methylbutyrate	0.11		
Ethyl Isovalerate	0.37		
Diethyl Maleate	0.75		
Ethyl Glycolate	1.91		

B. Typical Perhydrolase Peracid Generation Assay:

Perhydrolase is active over a wide pH and temperature range and accepts a wide range of substrates for acyl transfer. Acceptors include water (hydrolysis), hydrogen peroxide (perhydrolysis) and alcohols (classical acyl transfer). For perhydrolysis measurements enzyme was incubated in the buffer of choice at a specified temperature with a substrate ester in the presence of hydrogen peroxide. Typical substrates used to measure perhydrolysis include ethylacetate, triacetin, tributyrin, ethoxylated neodol acetate esters, and others. In addition, the wild type enzyme was found able to hydrolyze nitrophenylesters of short chain acids. The latter are convenient substrates to measure enzyme concentration. In some embodiments, peracid acid and acetic acid were measured by the ABTS or HPLC assays as described below. Nitrophenylester hydrolysis is also described below.

C. ABTS Assay (one milliliter):

This assay provides a determination of peracetic acid produced by perhydrolase. This protocol was adapted from Karst *et al.*, Analyst, 122:567–571 [1997]). Briefly, a 100 µL aliquot of solution to be analyzed was added to 1 mL 125 mM K^+citrate pH 5, 1 mM ABTS, 50 µM KI. Absorbance was measured at 420 nm for highest sensitivity. However, multiple additional wavelengths were sometimes used over the broad absorption spectrum of ABTS. Calibration curves were constructed based on known peracid concentration series.

D. HPLC (Model - Agilent 1100) Determination of Perhydrolase Reaction Products:

For determination of the ratio of perhydrolysis to hydrolysis of the perhydrolase reaction, perhydrolase reaction samples were quenched by acidification to a final concentration of 0.24% methanesulfonic acid, and the products were separated by reverse phase HPLC on a Dionex OA column (cat #062903; Dionex Corporation, Sunnyvale, CA). The mobile phase was 100 mM $NaPO_4$, pH 3.9 (buffer was prepared by titrating 100 mM Na_2PO_4 with methanesulfonic acid to pH 3.9) run under isocratic conditions at 30 °C. Detection was at 210 nm. Concentrations of products were calculated by comparison of the integrated peak areas against calibration standards.

E. Nitrophenylester Hydrolysis Kinetic Assay

Enzyme and substrate were incubated in 100 mM Tris/HCl pH 8.0 (or 50 mM $B(OH)_3$ pH 9.5 or another buffer). Absorbance at 402 nm was monitored. In some experiments, the assay was carried out in standard 1 mL cuvettes, while in other experiments, microtiter plate wells were used. The latter method was used for the screening of mutant libraries. Enzyme concentration was determined by comparison to standard curves obtained under the same reaction conditions.

F. Para-nitrophenylcaproate Hydrolysis Assay

The pNC6 substrate solution was prepared by mixing 1mM pNC6 (100 mM stock solution), 1 ml DMSO, 19 mls 100mM Phosphate (pH8), and glycerol to a final concentration of 10%. To assay samples, 10 µl of the cell lysate were added to 190 µl of the substrate solution, and assayed at 405 nm for 15 minutes in a spectrophotometer. The results are presented as the average of two experiments.

G. Para-nitrophenyl Acetate (pNA) Hydrolysis Assay

Aliquots of the lysed cell supernatant were diluted 1-100 in 100 mM phosphate buffer (pH 8). To assay the samples, 5 µl of the 1-100 diluted cell supernatant were placed into each well of a microtiter plate. Then, 195 µl of reaction buffer/substrate mix (1 mM pNA, 100 mM phosphate, pH 8, 10% glycerol) were added, and the absorbance rate at 405 nm was measured over 3 minutes (kinetics program, microtiter plate reader). The results are presented as the average of two experiments.

EXAMPLE 3

Assays Including Detergent Compositions

In this Example, assay systems used to screen for superior perhydrolase activity in detergents with particular substrates are provided. These assays include those that measure peracid degradation of perhydrolase, as well as the peracid synthesis activity of the enzyme.

Materials and Methods for Peracetic Acid Formation (PAF) and Peracetic Acid Degradation (PAD) Assays

This section provides the materials and methods used to screen for a superior perhydrolases in Ariel with C9E2OAC ester substrate

Materials:

Ariel Futur without bleach, perfume, or enzymes (P&G, Ariel "C")
C9E2OAc (P&G)
30% Hydrogen Peroxide (Sigma)
32% Peroxyacetic acid ("peracid", PAA)(Sigma cat#) MW = 76.05; 4.208M
Citric Acid, anhydrous MW=192.12
Potassium Hydroxide MW=56.11
ABTS (Sigma cat# A1888) MW=548.68
Potassium Iodide MW=166.0
Potassium Phosphate , mono and di-basic

Stock solutions:

Ariel detergent stock: Ariel Futur without bleach, perfume, or enzymes ("Ariel C") was dissolved in water to 6.72 g/L. It was stirred at room temp for 30 minutes, then allowed to settle. Then, it was divided into convenient aliquots and stored at 4°C, until used. When made and used fresh, the solution was filtered, instead of settled

100 mM C9E2OAc in Ariel detergent stock: First, 30 µl C9E2OAc was added to 970 µl Ariel detergent stock, using a positive displacement pipet. It was sonicated in a bath sonicator until a milky dispersion was formed (15-60 seconds). The dispersion was stable for about two hours. When used, 10 µl of dispersion per ml of reaction mix were used.

42 mM Peroxyacetic acid stock: Right before use, the Sigma 32% PAA solution was diluted 1:100 in water. Then 5.7 µl of the 42 mM stock per ml of reaction mix was added.

2 M hydrogen peroxide: One ml of 30% Sigma hydrogen peroxide was added to 3.41 ml water. This solution was prepared fresh, right before use. It was used at 10 µl per ml of reaction mix.

125 mM Citrate buffer pH 5.0: This was prepared to 24.0 grams per liter. It was made up in 800 ml, and titrated to pH 5.0 with 50% KOH. The volume was adjusted to 1 liter and stored at room temperature.

100 mM ABTS stock: This was prepared using 549 mg of ABTS in 10 ml of water. It was frozen at –80°C, in convenient aliquots in opaque Eppendorf tubes. The stock was stable indefinitely when kept frozen in the dark. ABTS will precipitate when thawed from –80°C but goes back into solution upon mixing. In use, 10 µl of ABTS stock was used per ml of ABTS reagent.

250 mM KI: This was prepared as 415 mg in 10 ml water. It was kept at 4°C. It was diluted to 25 mM working stock, and 2 ul of working stock was used per ml of ABTS reagent.

25 mM Potassium Phosphate buffer, pH 8.0:

Method:

The night prior to performance of the assays, the plates containing lysed cells that contain perhydrolase were checked to be sure that they were frozen twice. On the day of the assay, 30 to 45 minutes were allowed for the plates to thaw. The ABTS reagent was prepared and the Multidrop (Multidrop 384 instrument, ThermoElectron) to fill the detection plates with 200 µl per well. Store the filled plates covered at room temperature in the dark until needed. Dilutions of the standards were prepared so that when 20 µl of the diluted standard were added to the 180 µl of the reaction mix, the concentration in the well was 1 ppm. Four 4 two-fold serial dilutions were prepared to a set of six standards: 1, 0.5, 0.25, 0.125, and 0.0625 ppm final concentration in the wells.

To test, 20 µl of the standards were added to the thawed 1:10 dilution plate. The reaction mixtures were prepared and the Multidrop used to fill one reaction plate for each plate to be assayed (180µl/well). Note that the reaction mixtures are different for the PAF and PAD assays.

Peracid Hydrolysis (Peracid Degradation, PAD) Assay:

This assay measures the amount of peracetic acid remaining after a 100 minute incubation with enzyme in an Ariel detergent background. The amount of peracid remaining is detected by reacting an aliquot of the reaction mixture with the ABTS detection reagent.

In this assay, 20 µl enzyme samples from the thawed 1:10 dilution plate were transferred, one column at a time with an 8 channel pipetter, into the corresponding column of the pre-filled PAD reaction plate. A timer was started as soon as transfer occurred from the first column; subsequent columns were transferred at 15 second intervals (i.e., the last column was finished 2 min. 45 sec. after starting the first one). The plate was mixed for 30 seconds on the thermomixer (750 rpm, to avoid splashing). The plate was then transferred to a humidified chamber at 25°C. The plate was incubated for a total of 100 minutes from the time the first column of enzyme was added. At 100 minutes incubation, the reaction plate was removed from the incubator. Then, 20 ul aliquots of the reaction mixture were transferred to an ABTS reagent plate, in the same order and with the same 15 second time interval that the enzyme samples were originally added to the reaction plate. The ABTS plate was allowed to sit at room temperature for three minutes after the last column of reaction mixture was added. The plate was then read on the spectrophotometric plate reader at 420 and 740 nm.

Perhydrolysis (Peracid Formation, PAF) Assay

Multidrop Optimized Protocol: Screening for a Superior Perhydrolysis in Ariel with C9E2OAC Ester Substrate

The same materials and stock solutions described above for PAD were used in these experiments, as indicated below.

Method:

The methods were designed to assay 20 µl aliquots from a 1:100 dilution plate. The 20 µl 1:100 dilution assay plates were produced during the process of obtaining the protein concentrations and were stored at –80°C. The plates were thawed for about 30 to 45 minutes before use. Dilutions of the S54V standards were prepared, so that when 2 µl of the diluted standard are added to the 20 µl of the 1:100 diluted cell lysate, the concentration in the well was 0.1 ppm. Four two-fold serial dilutions were prepared to produced a set of six standards: 0.1, 0.05, 0.025, 0.0125, and 0.00625 ppm final concentration in the wells. Then, 2 ul of the standards were added to the thawed 20 ul 1:100 dilution assay plates in the wells indicated.

Perhydrolysis (Peracid formation, PAF) Assay:

This assay measures the amount of peroxyacetic acid that is produced in 10 minutes from the C9E2OAc substrate in an Ariel detergent background. The amount of peracid formed is detected after 10 minutes by reacting an aliquot of the reaction mixture with the ABTS detection reagent.

The Multidrop was used to deliver 180 µl/well of the PAF reaction mix to the prepared 1:100 dilution plate. The timer was started and the reaction plate was placed on the thermomixer, with the temperature set at 25°C. The plate was covered and the solutions mixed for 30 seconds at 750 rpm. The plate was then allowed to rest on the thermomixer without mixing, for a total of 10 minutes from the time the reaction mix was added. At 10 minutes, the Multidrop was used to add 20µl/well of the 10x ABTS reagent. The 10x reagent was a milky suspension. The thermomixer was used to briefly shake the plate. The ABTS reagent quickly went into solution. The plate was allowed to sit at room temperature for three minutes after the ABTS reagent was added. The plate was then read on the spectrophotometric plate reader at 420 nm.

EXAMPLE 4

Cloning of *Mycobacterium smegmatis* Perhydrolase

In this Example, the cloning of *M. smegmatis* perhydrolase is described. An enzyme with acyltransferase activity was purified from *Corynebacterium oxydans* (now *Mycobacterium parafortuitum* ATCC19686). Two peptide sequences were obtained from the purified protein. One peptide was determined by Edman degradation from cyanogen bromide cleavage of the purified enzyme using methods known in the art. The sequence of this peptide was determined to be KVPFFDAGSVISTDGVDGI (SEQ ID NO:3). The second peptide was analyzed using N-terminal sequencing and was found to have the GTRRILSFGDSLTWGWIPV (SEQ ID NO:4). A BLAST search against the

TIGR unfinished genome database identified a sequence of potential interest in *Mycobacterium smegmatis*, which is shown below:

MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLAQQLGADFEVIEEGLS
ARTTNIDDPTDPRLNGASYLPSCLATHLPLDLVIIMLGTNDTKAYFRRTPLDIALG
MSVLVTQVLTSAGGVGTTYPAPKVLVVSPPPLAPMPHPWFQLIFEGGEQKTTELA
RVYSALASFMKVPFFDAGSVISTDGVDGIHFTEANNRDLGVALAEQVRSLL (SEQ ID NO:2).

The corresponding DNA sequence of the gene is:

5'-
ATGGCCAAGCGAATTCTGTGTTTCGGTGATTCCCTGACCTGGGGCTGGGTCCC
CGTCGAAGACGGGGCACCCACCGAGCGGTTCGCCCCCGACGTGCGCTGGACC
GGTGTGCTGGCCCAGCAGCTCGGAGCGGACTTCGAGGTGATCGAGGAGGGAC
TGAGCGCGCGCACCACCAACATCGACGACCCCACCGATCCGCGGCTCAACGG
CGCGAGCTACCTGCCGTCGTGCCTCGCGACGCACCTGCCGCTCGACCTGGTG
ATCATCATGCTGGGCACCAACGACACCAAGGCCTACTTCCGGCGCACCCCGC
TCGACATCGCGCTGGGCATGTCGGTGCTCGTCACGCAGGTGCTCACCAGCGC
GGGCGGCGTCGGCACCACGTACCCGGCACCCAAGGTGCTGGTGGTCTCGCCG
CCACCGCTGGCGCCCATGCCGCACCCCTGGTTCCAGTTGATCTTCGAGGGCG
GCGAGCAGAAGACCACTGAGCTCGCCCGCGTGTACAGCGCGCTCGCGTCGTT
CATGAAGGTGCCGTTCTTCGACGCGGGTTCGGTGATCAGCACCGACGGCGTC
GACGGAATCCACTTCACCGAGGCCAACAATCGCGATCTCGGGGTGGCCCTCG
CGGAACAGGTGCGGAGCCTGCTGTAA-3' (SEQ ID NO:1)

Primers were designed based on the gene sequence to amplify and clone the gene. The primers used for amplification were:

MsRBSF: 5'-
CTAACAGGAGGAATTAACCATGGCCAAGCGAATTCTGTGTTTCGGTGATTCC
CTGACCT-3' (SEQ ID NO:5)

MspetBamR: 5'-
GCGCGCGGATCCGCGCTTACAGCAGGCTCCGCACCTGTTCCGCGAGGGCC
ACCCCGA-3' (SEQ ID NO:6)

The amplification of the gene was done by PCR using *Taq* DNA polymerase (Roche) per the manufacturer's instructions, with approximately 500 ng of chromosomal DNA from *Mycobacterium smegmatis* as the template DNA and the addition of 1% DMSO to the PCR reaction mix. Thirty picomoles of each of the primers MsRBSF and MspetBamR were added to the mix. The amplification cycle was: 30 cycles of (95°C for 1 min, 55°C for 1 min, 72°C for 1 min).

The fragments obtained from the PCR reaction were separated on a 1.2% agarose gel and a single band of the expected size of 651 bp (coding sequence and stop codon) was identified. This band was cloned directly into the pCR2.1 TOPO cloning vector (Invitrogen) and transformed into *E. coli* Top 10 cells (Invitrogen) with selection on L agar (10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl, 20 g/l agar) containing 100 micrograms/ml carbenicillin and X-gal (20 micrograms/ml, Sigma-Aldrich) for blue/white selection and incubated overnight at 37°C. Five white colonies were analyzed for the presence of the PCR fragment. Each colony was used to inoculate 5 mls of L broth (L agar without the addition of agar) containing 100 micrograms/ml carbenicillin and the cultures were grown overnight at 37°C with shaking at 200 rpm. Plasmid DNA was purified from the cultures using the Quikspin kit (Qiagen). The presence of the correct fragment was determined by restriction enzyme digest with *Eco*R1 to release the fragment, and sequencing using primers supplied by the pCR2.1 manufacturer (Invitrogen). The correct plasmid was designated pMSATNcoI (*See*, Figure 12, for the map of this plasmid)). The sequence of this plasmid is provided below

agcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
cgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgtt
gtgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgccaagctatttaggtgacactatagaat

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actcaagctatgcatcaagcttggtaccgagctcggatccactagtaacggccgccagtgtgctggaattcgcccttctaacagga
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gagcggttcgcccccgacgtgcgctggaccggtgtgctggcccagcagctcggagcggacttcgaggtgatcgaggagggac
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cgaattctgcagatatccatcacactggcggccgctcgagcatgcatctagagggcccaattcgccctatagtgagtcgtattaca
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gcgcgacccggccggcaactgcgtgcacttcgtggccgaggagcaggactgacacgtgctaaaacttcatttttaatttaaaagg
atctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaa
gatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgttt
gccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagcc
gtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtgg
```

cgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacggggggttcgtg
cacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccg
aagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaac
gcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatg
gaaaaacgccagcaacgcggcctttttacggttcctgggcttttgctggccttttgctcacatgttctttcctgcgttatcccctgattct
gtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcga
ggaagcggaag (SEQ ID NO:13)

Construction of Perhydrolase T7 Expression Plasmid

The primer pair used to create pMSATNco1 was also used to create an *Nco*I site (CCATGG) in which the ATG is the start codon of the acyltransferase gene and a *Bam*H1 (GGATCC) just after the TAA stop codon. The plasmid pMSATNco1 was digested with *NcoI/Bam*H1 as recommended by the manufacturer (Roche) and the 658 bp fragment containing the perhydrolase gene was purified using standard procedures known in the art (*e.g.*, Sambrook *et al.*). The fragment was ligated using standard procedures known in the art (*e.g.*, Sambrook *et al.*) into the T7 promoter expression plasmid, pET16b (Novagen), also digested with *NcoI/Bam*H1. The ligation reaction was transformed by standard procedures into *E. coli* Top 10 cells (Invitrogen) and selected on L agar containing 100 micrograms/ml carbenicillin overnight at 37°C. Ten colonies were picked from the several transformants and used to inoculate 5 ml of LB containing 100 micrograms/ml carbenicillin. Cultures were grown overnight at 37°C with shaking at 200 rpm. Plasmid DNA was purified from the cultures using the Qiagen Quikspin kit (Qiagen). The presence of the correct fragment was determined by restriction enzyme digest with *NcoI/Bam*H1 as directed by the manufacturer. The correct plasmid was designated pMSATNcoI-1 (*See*, Figure 13, for the map of this plasmid). In this Figure, the following elements are indicated--LacI: gene encoding the LacI repressor protein, located at bp1455-2534, ori: plasmid origin of replication at bp 4471, bla: The β-lactamase gene located at bp 6089-5232; T7 promoter: located at bp1068-1052; T7 terminator: located at bp 259-213, *per*: the *M. smegmatis* perhydrolase gene located at 981-334. The sequence of this plasmid is provided below:

ttctcatgtttgacagcttatcatcgataagctttaatgcggtagtttatcacagttaaattgctaacgcagtcaggcaccgtgtatgaa
atctaacaatgcgctcatcgtcatcctcggcaccgtcaccctggatgctgtaggcataggcttggttatgccggtactgccgggcct
cttgcgggatatccggatatagttcctcctttcagcaaaaaacccctcaagacccgtttagaggccccaaggggttatgctagttatt
gctcagcggtggcagcagccaactcagcttcctttcgggctttgttagcagccggatccgcgcgcttacagcaggctccgcacct
gttccgcgagggccaccccgagatcgcgattgttggcctcggtgaagtggattccgtcgacgccgtcggtgctgatcaccgaac
ccgcgtcgaagaacggcaccttcatgaacgacgcgagcgcgctgtacacgcgggcgagctcagtggtcttctgctcgccgccc
tcgaagatcaactggaaccaggggtgcggcatgggcgccagcggtggcggcgagaccaccagcaccttgggtgccgggtac
gtggtgccgacgccgcccgcgctggtgagcacctgcgtgacgagcaccgacatgcccagcgcgatgtcgagcggggtgcgc
cggaagtaggccttggtgtcgttggtgcccagcatgatgatcaccaggtcgagcggcaggtgcgtcgcgaggcacgacggcag
gtagctcgcgccgttgagccgcggatcggtggggtcgtcgatgttggtggtgcgcgcgctcagtccctcctcgatcacctcgaag
tccgctccgagctgctgggccagcacaccggtccagcgcacgtcgggggcgaaccgctcggtgggtgccccgtcttcgacgg
ggacccagccccaggtcagggaatcaccgaaacacagaattcgcttggccatggtatatctccttcttaaagttaaacaaaattattt
ctagaggggaattgttatccgctcacaattcccctatagtgagtcgtattaatttcgcgggatcgagatctcgatcctctacgccgga
cgcatcgtggccggcatcaccggcgccacaggtgcggttgctggcgcctatatcgccgacatcaccgatggggaagatcgggc
tcgccacttcgggctcatgagcgcttgtttcggcgtgggtatggtggcaggccccgtggccgggggactgttgggcgccatctcc
ttgcatgcaccattccttgcggcggcggtgctcaacggcctcaacctactactgggctgcttcctaatgcaggagtcgcataaggg
agagcgtcgagatcccggacaccatcgaatggcgcaaaacctttcgcggtatggcatgatagcgcccggaagagagtcaattca
gggtggtgaatgtgaaaccagtaacgttatacgatgtcgcagagtatgccggtgtctcttatcagaccgtttcccgcgtggtgaacc
aggccagccacgtttctgcgaaaacgcgggaaaaagtggaagcggcgatggcggagctgaattacattcccaaccgcgtggca
caacaactggcgggcaaacagtcgttgctgattggcgttgccacctccagtctggccctgcacgcgccgtcgcaaattgtcgcgg
cgattaaatctcgcgccgatcaactgggtgccagcgtggtggtgtcgatggtagaacgaagcggcgtcgaagcctgtaaagcgg
cggtgcacaatcttctcgcgcaacgcgtcagtgggctgatcattaactatccgctggatgaccaggatgccattgctgtggaagct
gcctgcactaatgttccggcgttatttcttgatgtctctgaccagacacccatcaacagtattattttctcccatgaagacggtacgcg
actgggcgtggagcatctggtcgcattgggtcaccagcaaatcgcgctgttagcgggcccattaagttctgtctcggcgcgtctgc
gtctggctggctggcataaatatctcactcgcaatcaaattcagccgatagcggaacgggaaggcgactggagtgccatgtccgg
ttttcaacaaaccatgcaaatgctgaatgagggcatcgttcccactgcgatgctggttgccaacgatcagatggcgctgggcgcaa
tgcgcgccattaccgagtccgggctgcgcgttggtgcggatatctcggtagtgggatacgacgataccgaagacagctcatgtta
tatcccgccgttaaccaccatcaaacaggattttcgcctgctggggcaaaccagcgtggaccgcttgctgcaactctctcagggcc
aggcggtgaagggcaatcagctgttgcccgtctcactggtgaaaagaaaaaccaccctggcgcccaatacgcaaaccgcctctc
cccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaat
gtaagttagctcactcattaggcaccgggatctcgaccgatgcccttgagagccttcaacccagtcagctccttccggtgggcgcg
gggcatgactatcgtcgccgcacttatgactgtcttctttatcatgcaactcgtaggacaggtgccggcagcgctctgggtcatttc
ggcgaggaccgctttcgctggagcgcgacgatgatcggcctgtcgcttgcggtattcggaatcttgcacgccctcgctcaagcctt
cgtcactggtcccgccaccaaacgtttcggcgagaagcaggccattatcgccggcatggcggccgacgcgctgggctacgtctt
gctggcgttcgcgacgcgaggctggatggccttccccattatgattcttctcgcttccggcggcatcgggatgcccgcgttgcagg
ccatgctgtccaggcaggtagatgacgaccatcagggacagcttcaaggatcgctcgcggctcttaccagcctaacttcgatcac
tggaccgctgatcgtcacggcgatttatgccgcctcggcgagcacatggaacgggttggcatggattgtaggcgccgccctatac
cttgtctgcctccccgcgttgcgtcgcggtgcatggagccgggccacctcgacctgaatggaagccggcggcacctcgctaacg
gattcaccactccaagaattggagccaatcaattcttgcggagaactgtgaatgcgcaaaccaacccttggcagaacatatccatc

gcgtccgccatctccagcagccgcacgcggcgcatctcgggcagcgttgggtcctggccacgggtgcgcatgatcgtgctcct
gtcgttgaggacccggctaggctggcggggttgccttactggttagcagaatgaatcaccgatacgcgagcgaacgtgaagcga
ctgctgctgcaaaacgtctgcgacctgagcaacaacatgaatggtcttcggtttccgtgtttcgtaaagtctggaaacgcggaagtc
agcgccctgcaccattatgttccggatctgcatcgcaggatgctgctggctaccctgtggaacacctacatctgtattaacgaagcg
ctggcattgaccctgagtgatttttctctggtcccgccgcatccataccgccagttgtttaccctcacaacgttccagtaaccgggca
tgttcatcatcagtaacccgtatcgtgagcatcctctctcgtttcatcggtatcattacccccatgaacagaaatcccccttacacgga
ggcatcagtgaccaaacaggaaaaaaccgcccttaacatggcccgctttatcagaagccagacattaacgcttctggagaaactc
aacgagctggacgcggatgaacaggcagacatctgtgaatcgcttcacgaccacgctgatgagctttaccgcagctgcctcgcg
cgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtctgtaagcggatgccgggagc
agacaagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggggcgcagccatgacccagtcacgtagcgatagcgga
gtgtatactggcttaactatgcggcatcagagcagattgtactgagagtgcaccatatatgcggtgtgaaataccgcacagatgcgt
aaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggta
tcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagca
aaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacg
ctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgtt
ccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctca
gttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactat
cgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgta
ggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagcc
agttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagca
gattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgtta
agggattttggtcatgagattatcaaaaaggatcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatatat
gagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcctga
ctccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcac
cggctccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatcca
gtctattaattgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctgcaggcatcgtgg
tgtcacgctcgtcgtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaa
agcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccgcagtgttatcactcatggttatggcagcactgcataatt
ctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatagtgtatgcggcgaccg
agttgctcttgcccggcgtcaacacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttcttcg
gggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatctttta
ctttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaataagggcgacacggaaatgttga
atactcatactcttcctttttcaatattattgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaata
aacaaataggggttccgcgcacatttccccgaaaagtgccacctgacgtctaagaaaccattattatcatgacattaacctataaaa
ataggcgtatcacgaggccctttcgtcttcaagaa (SEQ ID NO:131)

This plasmid was transformed into the *E. coli* strain BL21(λDE3)pLysS (Novagen), which contains the gene encoding the T7 RNA polymerase, with selection on

LA containing 100 micrograms/ml carbenicillin. Cells were grown overnight at 37°C. One transformant was selected and the strain was designated MSATNco1.

Production of Perhydrolase in MSATNco1-1

Production of perhydrolase was done in cell culture. For example, 5 ml of LB with carbenicillin at a concentration of 100 micrograms/ml was inoculated with a single colony of MSATNco1 and grown overnight at 37°C with shaking at 200 rpm. This culture was used to inoculate 100 ml of LB with carbenicillin at a concentration of 100 micrograms/ml (in a 250 ml baffled flask) to an OD_{600} of 0.1. The cultures were grown at 30°C with shaking at 200 rpm until they reached an OD_{600} of 0.4. The expression of the perhydrolase gene was then induced by the addition of 100 micromolar IPTG and the incubation continued overnight. Cultures were harvested by centrifugation (10 min at 7000 rpm, Sorvall SS34 rotor), the supernatant was removed and the pellets washed in 50 mM KPO_4, pH 6.8. The cells were centrifuged again, the supernatants removed and the wet weight of the cells was determined. The cells were resuspended in 100 mM KPO_4 in a volume that was 4x the wet weight. The resuspended cells were frozen at –70°C. The cells were thawed and lysed in a French Pressure cell using standard procedures known in the art. The purification steps and assessment methods are provided in Example 1. Figure 6 provides a purification table showing the enzyme activity of the perhydrolase of the present invention through various steps in the purification process.

***M. smegmatis* Perhydrolase is in an Operon**

In additional experiments, it was determined that the *M. smegmatis* perhydrolase is part of an operon. The gene (*phd*) is the first gene in an operon that contains at least 2 genes, including *phd*, that are separated by 10 bp (GGCTGGGGGC [SEQ ID NO:7]) not including the TAA stop codon of *phd*. It is also possible that there are three genes in the operon, with the third being either 48 bp or 61 bp to the next ORF (open reading frame).

The latter two candidate genes have no significant homology to proteins in the database.

A putative promoter was identified for *M. smegmatis phd* operon, TTGGGC (-35) SP (18) CCAGAT by sequence analysis and comparison with known *M. smegmatis* promoters (*See e.g.,* Salazar *et al.,* Microbiol., 149:773-784 [2003]). It is not intended that the present invention be limited to any particular promoter and/or construct design, as it is contemplated that other promoters and construct designs will find use in the present invention.

The second gene in the *phd* operon encodes a protein (putative PBP-3) with the sequence:

mhlrpaltwllvvglfisvvgcssspdpadrfsafaealgrkdaaaaaaqtsdpaaaeaaitamlagmgdaanvsvaaepee
gddagatlkytwtwgegrdfgydttataaksgddwlitwsptvlhrdltpdlrfqysedselqtpvldrtgqplmtwqtvgvit
verahpesaaplaallapfdptttesvtaqlnsttddrvtvmklreddlgqvrdqlaqipgvtvreqgelltadrqlsspaisgld
elwhdritanagwsvylvdadgapaqqltstppkdtgpvrttldlrmqllaqqavaketrpavvvaisgstggilaaaqnpaa
dpqgaiafsglyppgstfktittaaaldaglatpdtpvacpgeltienrtipnddnfdlgtvplssafshscntsmaalsdelppn
altdmakdfgigvdfmvpglttvtgrvpnadnaaqrvengigqgtvtvspfglavaeaslahgstilptlvdgekttadtpsvp
lppnitdalrammrgtvtegtatalsdipdlggktgtaefgdnthshgwfagiagdiafatlvvggdssapavaisgdflrpala
g (SEQ ID NO:9)

The corresponding DNA sequence of the gene encoding the putative PBP-3:

atgcacttacgtcccgctctgacgtggctcctggttgtcggtctgttcatatcggtcgtcggatgttcgtcgtccccggatccggccg
accggttctcggcgttcgccgaggcgctgggccgcaaggatgcggccgcggcggccgcccagaccagcgatccggcggcc
gcggaggcggccatcaccgcgatgctggccgggatgggcgacgccgcgaacgtctcggtggccgccgaacccgaggaagg
cgacgacgcgggcgcgacgctgaagtacacgtggacctggggtgagggccgcgacttcggctacgacaccaccgcgacggc
ggccaaatccggtgacgactggctgatcacctggtcccccaccgtgttgcaccgcgacctcaccccggatctgcgcttccagtac
agcgaggacagcgaattgcagaccccggtgctcgaccgcaccggccagccgttgatgacatggcagaccgtcggtgtcatcac
tgtcgaacgcgcacatccggagtcggccgcaccgctcgccgccctgctggcgcccttcgatccgaccaccaccaccgaatcgg
tcaccgcacaactcaattcgacgaccgatgaccgcgtgacggtgatgaagctgcgcgaggacgatctgggtcaggtgcgcgat
cagctcgcgcagatccccggcgtgaccgtgcgtgagcagggtgagctgctcaccgccgaccggcagctgtcctcgcccgccat
cagcggcctggacgagctgtggcacgaccggatcaccgccaacgcgggctggtcggtgtacctggtcgacgccgacggtgca
cccgcacaacagctcacgtccacgccgcccaaggacaccgggcccgtgcgcaccacgctggacctgcgcatgcaactgctcg
cgcagcaggccgtggccaaggagacccgcccggccgtggtggtcgcgatctccggatcgaccgggggcatcctggccgccg
cacagaacccggccgccgatccgcaaggtgcgatcgcgttttcgggcctgtacccgccggggtcgacgttcaagaccatcacc
acggcggcagccctcgacgcgggcctggccaccccggacacaccggtggcctgcccgggtgagctcaccatcgagaaccgc
acgatccccaacgacgacaacttcgacctgggcaccgtgccgttgtcgtcggcgttctcgcactcctgcaacaccagcatggcc
gccctgtccgacgagctgccgcccaacgcactgaccgacatggcaaaggacttcgggatcggcgtcgacttcatggtgcccgg

cctgaccaccgtgaccggccgtgtccccaacgccgacaacgccgcccagcgtgtcgagaacggcatcggccagggcaccgt
gaccgtcagcccgttcggcctcgccgtcgccgaggccagcctggcgcacggttcgacgatcctgccgacgctggtcgacggc
gagaagaccacggccgacaccccgtcggtgccgttgccgcccaacatcaccgacgcgctgcgcgcgatgatgcgcggaacg
gtcaccgagggcacggccaccgcgttgagcgacatccccgacctgggcggcaagaccggcacggcggaattcggcgacaac
acgcactcgcacggctggttcgcgggcatcgcgggcgacatcgcgttcgcgacgctggtggtcggcggcgactcgtcggcac
cggccgtcgcgatctcaggagacttcctgcgccccgcgctcgccggctag (SEQ ID NO:8).

A standard BLAST search against the protein database identified homology with several penicillin binding proteins, class 3 (PBP-3). By sequence alignment and comparison to literature (*e.g.*, Goffin and Ghysen, Microbiol. Mol. Biol. Rev., 66:702-38 [2002]) the PBP was found to contain the required bar codes (conserved protein sequences that define a class of proteins) to place it in the SxxK superfamily of acyl transferases, with a C-terminal domain acyl transferase and an N-terminal domain of unknown function, but with homology to the Pen^r (*i.e.*, penicillin resistant) protein fusions of class B-like II and III. This penicillin binding protein acyl transferase domain does not share significant homology with the perhydrolase of the present invention, although it does share homology with Co-A dependent acyl transferases known in the art. The amino acid sequence is provided below.

MHLRPALTWLLVVGLFISVVGCSSSPDPADRFSAFAEALGRKDAAAAAAQTSDP
AAAEAAITAMLAGMGDAANVSVAAEPEEGDDAGATLKYTWTWGEGRDFGYDT
TATAAKSGDDWLITWSPTVLHRDLTPDLRFQYSEDSELQT**PVLDRTG**QPLMTWQ
TVGVITVERAHPESAAPLAALLAPFDPTTTTESVTAQLNSTTDDRVTVMKLREDD
LGQVRDQLAQIPGVTVREQGELLTADRQLSSPAISGLDELWHDRITANAGWSVYL
VDADGAPAQQLTSTPPKDTGPVRT**TLDLRMQ**LLAQQAVAKETRPAVVVAISGS
TGGILAAAQNPAADPQGAIAFSGLYPPG**STFK**TITTAAALDAGLATPDTPVACPG
ELTIENRTIPNDDNFDLGTVPLSSAFSH**SCN**TSMAALSDELPPNALTDMAKDFGIG
VDFMVPGLTTVTGRVPNADNAAQRVENGIGQGTVTVSPFGLAVAEASLAHGSTI
LPTLVDGEKTTADTPSVPLPPNITDALRAMMRGTVTEGTATALSDIPDLGG**KTGT**
AEFGDNTHSHGWFAGIAGDIAFATLVVGGDSSAPAVAISGDFLRPALAG (SEQ ID
NO:10)

The family-identifying bar codes provided in the above review were: (19) V (20)

G/A (140) PVxDRTG (142) TxDx3Q (22) TGGxLAx4PaxDP (13) SxxK (51) SCN (131) KTG (50) marked in bold letters in the above sequence. The letters represent the amino acid sequence defining the bar code; the numbers in brackets are the intervening number of amino acids between the particular bar codes; "x" represents any amino acid, (*i.e.*, the amino acids are not conserved within the bar code but the number of amino acids (*e.g.*, x3 corresponding to 3 intervening amino acids) is conserved). Based on these results and other data, as described herein, it is clear that the perhydrolase of the present invention represents a unique enzyme class.

EXAMPLE 5

Expression of the Perhydrolase in *P. citrea*

In this Example, methods used to express the perhydrolase in *P. citrea* are described. The plasmid pMSATNcoI was transformed into *P. citrea* by electroporation using the method essentially as known in the art (*See e.g., Sambrook et al., supra*) except that all cultures and recovery were done at 30°C. The transformants were plated on L agar + carbenicillin (200 µg/ml) and incubated overnight at 30°C. Three transformants were picked for analysis. Each colony was used to inoculate a 30 ml culture of LB + carbenicillin (200 µg/ml) and grown overnight at 30°C with shaking at 200 rpm. The cells were pelleted by centrifugation, washed one time in 50 mM phosphate buffer pH 7.2, and finally resuspended in 4x the wet cell weight of 100 mM phosphate buffer pH 8.0. The cells were lysed by treatment with lysozyme (2 µl of a 10 mg/ml solution per one ml of *P. citrea* culture) at 37°C for one hour. The cell debris was pelleted at 13,000 rpm in a microfuge for 5 min. The resulting supernatant was used for further analysis in SDS-PAGE and Western blots, as well as assays for enzyme activity.

SDS-PAGE analysis was carried out as known in the art (*See e.g.*, Sambrook *et al., supra*) on the supernatants. Detection of the perhydrolase protein by Western blot was done using an anti-perhydrolase polyclonal anti-sera (prepared from purified perhydrolase protein by Covance). The blot was developed as per manufacturer's suggestions using the ECL plus kit (Amersham).

The enzymatic activity of the expressed perhydrolase was detected by the pNB (para-nitrophenylbutyrate) assay as described in Example 1, herein. The results are provided in the

Table 5-1. Enzymatic Activity of Perhydrolase Expressed by *P. citrea*

Clone	OD405	Rate	Concentration (mg/liter)
P. citrea/ pMSATNcoI	3.1129	0.47948	7.1922
Control (*P. citrea*)	2.6187	-9.8312	0

The SDS-PAGE and Western blot results, as well as the assay results indicated that the perhydrolase is expressed by *P. citrea* and is active.

EXAMPLE 6

Expression of the Perhydrolase in *Bacillus subtilis*

The perhydrolase was expressed intracellularly in *B. subtilis*. A variety of promoters find use in this embodiment, including but not limited to pSPAC, pAprE, pAmyE, pVeg, pHpaII. In some embodiments, the construct is present on a replicating plasmid (*e.g.*, pBH1), while in other embodiments, it is integrated into the chromosome in one or more copies. Examples of sites for integration include, but are not limited to the *aprE*, the *amyE*, the *veg* or the *pps* regions. Indeed, it is contemplated that other sites known to those skilled in the art will find use in the present invention.

A. Intracellular Expression of the Perhydrolase in *Bacillus subtilis* From

a Replicating Plasmid

B. subtilis expresses a lipase/esterase encoded by the gene *pnbA* that hydrolyzes the pNB substrate used to detect activity of the perhydrolase. To identify *B. subtilis* strains expressing the perhydrolase after transformation with replicating or integrating plasmids the *pnbA* gene (the entire coding sequence) was first deleted from the desired host using the *lox*P cassette deletion method described in WO 03/083125, herein incorporated by reference. It is also noted that other strains of *Bacillus* may contain one or more lipases/esterases capable of hydrolyzing the pNB or other substrate used as an indicator for perhydrolase activity. In some embodiments, for optimal expression and/or activity detection it is necessary to delete one or more of the lipases/esterases from the hosts. The *Bacillus subtilis* strain used in this Example has the genotype *Bacillus subtilis* comK *pnbA* (*pnbA*loxP-spec, *aprE, nprE, degUHy32, oppA, spoIIE3501* and will be referred to as "*B. subtilis pnbA*" (*See e.g.,* WO 03/083125, supra).

In these experiments, a consensus *Bacillus* ribosome binding site (RBS) was used. It is not intended that the consensus RBS be the only sequence used for expression, as a non-consensus RBS also finds use in the present invention. The RBS of pMSATNcoI (*See*, Example 4) was changed to a *Bacillus* consensus RBS from the 16S rRNA (5'-ATAAGGAGGTGATC -3' [SEQ ID NO:132]) of *B. subtilis* and a *Hin*dIII site was added to the 5' end of the RBS by PCR using a primer (502rbsforward primer) containing the desired changes. The reaction was carried out using an MJ Research PCR machine with 30 cycles of (1 min at 95°C, 1 min at 55°C, and 1 min at 72°C). Template DNA (pMSATrbs) was added to a 50 µl reaction (10 ng) and 10 picomoles of each primer were used.

The PCR-generated *phd* cassette was cloned into the PCR cloning vector, pCR-Script CM (Stratagene) and transformed into *E. coli* Top10 cells (Invitrogen) to make pAH502R. The complete sequence of this plasmid is provided below.

ctaaattgtaagcgttaatattttgttaaaattcgcgttaaatttttgttaaatcagctcattttttaaccaataggccg
aaatcggcaaaatcccttataaatcaaaagaatagaccgagatagggttgagtgttgttccagtttggaacaagagtcca
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tttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaataggggttccgcgcac
atttccccgaaaagtgccac (SEQ ID NO:133)

Transformants were selected on L agar containing 100 μg/ml carbenicillin. The construct was confirmed by sequencing and biochemical assays (*e.g.*, pNB activity assay)

Primer set for pAH502R construction:

502rbsForward primer:
5'- ccaagcttaaggaggtgatctagaattccatggccaagcgaattctgtgtttcg-3' (SEQ ID NO:134)

502Reverse Primer:
5'- ggggatccttttacagcaggctccgcacct-3' (SEQ ID NO:135)

The *Hind*III-RBS-phd-*BamH* I DNA fragment from pAH502R was cloned into the pSPAC containing vector, pMUTIN4 (*See,* Vagner *et al.*, Microbiol., 144, 3097-3104 [1998]) creating the construct pAH503. The complete sequence of pAH503 is provided below:

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tcagaacgctcggttgccgccgggcgtttttatgcagcaatggcaagaacgttgctctaga (SEQ ID NO:136)

The construction of pAH503 was confirmed by RFLP and pNB activity assays. The pSPAC-RBS-phd DNA cassette was isolated as a *Bgl*II/*Sma*I digest and then subcloned into the replicating plasmid pBH1, digested with *Bam*H1/*Eco*RV (*See e.g.*, EP 0275509) to create pAH505 (*See*, Figure 14). The complete sequence of the plasmid is provided below.

gatcttccaagatatcctaacagcacaagagcggaaagatgttttgttctacatccagaacaacctctgctaaaattcctgaaaaattt
tgcaaaaagttgttgactttatctacaaggtgtggcataatgtgtggaattgtgagcgctcacaattaagcttaaggaggtgatctag
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ggttcgcccccgacgtgcgctggaccggtgtgctggcccagcagctcggagcggacttcgaggtgatcgaggagggactgag
cgcgcgcaccaccaacatcgacgaccccaccgatccgcggctcaacggcgcgagctacctgccgtcgtgcctcgcgacgcac
ctgccgctcgacctggtgatcatcatgctgggcaccaacgacaccaaggcctacttccggcgcaccccgctcgacatcgcgctg
ggcatgtcggtgctcgtcacgcaggtgctcaccagcgcgggcggcgtcggcaccacgtacccggctcccaaggtgctggtggt
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gaatccacttcaccgaggccaacaatcgcgatctcggggtggccctcgcggaacaggtgcggagcctgctgtaaaaggatccc
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atgaagatggattttctattattgcaatgtggaattgggaacggaaaaattattttattaaagagtagttcaacaaacgggccagtttgt
tgaagattagatgctataattgttattaaaaggattgaaggatgcttaggaagacgagttattaatagctgaataagaacggtgctctc
caaatattcttatttagaaaagcaaatctaaaattatctgaaaagggaatgagaatagtgaatggaccaataataatgactagagaag
aaagaatgaagattgttcatgaaattaaggaacgaatattggataaatatggggatgatgttaaggctattggtgtttatggctctcttg
gtcgtcagactgatgggccctattcggatattgagatgatgtgtgtcatgtcaacagaggaagcagagttcagccatgaatggaca
accggtgagtggaaggtggaagtgaattttgatagcgaagagattctactagattatgcatctcaggtggaatcagattggccgctt
acacatggtcaatttttctctattttgccgatttatgattcaggtggatacttagagaaagtgtatcaaactgctaaatcggtagaagcc
caaacgttccacgatgcgatttgtgcccttatcgtagaagagctgtttgaatatgcaggcaaatggcgtaatattcgtgtgcaagga
ccgacaacatttctaccatccttgactgtacaggtagcaatggcaggtgccatgttgattggtctgcatcatcgcatctgttatacgac
gagcgcttcggtcttaactgaagcagttaagcaatcagatcttccttcaggttatgaccatctgtgccagttcgtaatgtctggtcaac
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gatgtgtcaaaacgcataccattttgaacgatgacctctaataattgttaatcatgttggttacgtatttattaacttctcctagtattagta
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ttgccagtcggggatattaaaaagagtataggtttttattgcgataaactaggtttcactttggttcaccatgaagatggattcgcagtt

ctaatgtgtaatgaggttcggattcatctatgggaggcaagtgatgaaggctggcgctctcgtagtaatgattcaccggtttgtacag
gtgcggagtcgtttattgctggtactgctagttgccgcattgaagtagagggaattgatgaattatatcaacatattaagcctttgggc
attttgcaccccaatacatcattaaaagatcagtggtgggatgaacgagactttgcagtaattgatcccgacaacaatttgattagctt
ttttcaacaaataaaaagctaaaatctattattaatctgttcagcaatcgggcgcgattgctgaataaaagatacgagagacctctctt
gtatctttttatttgagtggttttgtccgttacactagaaaaccgaaagacaataaaaattttattcttgctgagtctggctttcggtaag
ctagacaaaacggacaaaataaaaattggcaagggtttaaaggtggagatttttgagtgatcttctcaaaaaatactacctgtccct
tgctgatttttaaacgagcacgagagcaaaacccccctttgctgaggtggcagagggcaggtttttttgtttctttttctcgtaaaaaa
aagaaaggtcttaaaggttttatggttttggtcggcactgccgacagcctcgcaggacacacactttatgaatataaagtatagtgtg
ttatactttacttggaagtggttgccggaaagagcgaaaatgcctcacatttgtgccacctaaaaaggagcgatttacatatgagttat
gcagtttgtagaatgcaaaaagtgaaatcagggg (SEQ ID NO:137)

The ligation mixture for pAH505 was transformed into *Bacillus subtilis pnbA*. Correct transformants were verified by RFLP and sequencing of isolated plasmid DNA. One transformant was selected for analysis (*B. subtilis pnbA*/pAH505).

Expression of the perhydrolase in *Bacillus* was assayed using the pNB Activity Assay described herein, after growth of the desired strain in shake flask. The data showed that the perhydrolase was expressed in *B. subtilis pnbA*.

B. Intracellular Expression of the Perhydrolase in *B. subtilis pnbA* by Integration into the Chromosome

An additional construct useful to determine expression of the perhydrolase (*act*) gene integrated into the chromosome of *B. subtilis pnbA* involved use of the *spoVG* promoter, which was found to drive expression of the perhydrolase gene in a non-replicating (*i.e.*, integrating plasmid). In some embodiments, one site of integration is the *aprE* region of *B. subtilis*, although it is intended that integration occur at any suitable site. Indeed, it is not intended that the present invention be limited to this specific site nor this specific promoter, as various other suitable sites and promoters find use in the present invention.

The configuration of the promoter/gene at the *aprE* locus in the chromosome of *Bacillus subtilis* was as follows:

pAprE-aprE first 7 codons-translation stop-pSpoVG-ATG-perhydrolase gene from second codon

The clone was constructed as described below. The primers used were:

Up5'F
caggctgcgcaactgttgggaag (SEQ ID NO:138)

FuaprEAct34R
agtagttcaccaccttttccctatataaaagcattagtgtatcaatttcagatccacaattttttgcttctcactctttac (SEQ ID NO:139)

FuaprEAct4F
Aattgatacactaatgcttttatatagggaaaaggtggtgaactactatggccaagcgaattctgtgtttcggtg (SEQ ID NO:140)

*Bsm*I-DnAct504R
gtgagaggcattcggatccttttacagcaggctccg (SEQ ID NO:141)

PCR fusion is a technique well known in the art, in which two or more fragments of DNA are generated either by restriction digest or by PCR amplification. The fragments have overlapping segments, usually at least 18 bases long. In the instance that two fragments are used, the 3' end of fragment #1 has an overlapping sequence with the 5' end of fragment #2. The two fragments are used as template in a PCR reaction in which the primer set used hybridizes to the 5' end of fragment #1 (forward primer) and the 3' end of fragment #2 (reverse primer). During the amplification, the two regions of overlap hybridize forming a single template from which the two primers can amplify a full length fragment, a "fusion" of fragments #1 and #2. Multiple fragments of any length can be used in such a reaction, limited only by the ability of the chosen polymerase to amplify long DNA pieces.

In the current example, the above construct was made by PCR fusion of two PCR products the above construct was made by PCR fusion of two PCR products. The first was a construct with the *spoVG* promoter added upstream of the *phd* gene. The second was the *aprE* promoter and first 7 codons of *aprE*, followed by a stop codon. Regions of 20 bp overlap were added on the 5' and 3' ends of the products respectively, to allow the PCR fusion reaction. The primer set FuaprEAct4F/BsmI-DnAct504R was used to amplify the perhydrolase gene from pAH505 as described above, which added the *spoVG* promoter sequence (contained within the primer) to the 5' end of the gene and changed the start codon from ATG to GTG. To create the second product (pAprE plus the first 7 codons of *aprE*) for the fusion, the primer set Up5'F/FuaprEAct34R was used to amplify a fragment from pBSFNASally. Figure 15 provides a map of this plasmid. The complete sequence of pBSFNASally is provided below.

ctaaattgtaagcgttaatattttgttaaaattcgcgttaaatttttgttaaatcagctcattttttaaccaataggccgaaatcggcaaaat
cccttataaatcaaaagaatagaccgagatagggttgagtgttgttccagtttggaacaagagtccactattaaagaacgtggactc
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ctcttcgctattacgccagctggcgaaagggggatgtgctgcaaggcgattaagttgggtaacgccagggttttcccagtcacgac
gttgtaaaacgacggccagtgagcgcgcgtaatacgactcactatagggcgaattggagctccaccgcggtggcggccgctcta
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atgtcatttctgaaaaaggcgggaaagtgcaaaagcaattcaaatatgtagacgcagcttcagctacattaaacgaaaaagctgta
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gataaaatcatctcaaaaaaatgggtctactaaaatattattccatctattacaataaattcacagaatagtcttttaagtaagtctactct
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gtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagc
gtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccc
gttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagc
cactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactaga
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gctggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacgg
ggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatcttcacctagatccttttaaat
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gtgttatcactcatggttatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaa
ccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacatagcag
aactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaa
cccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgca
aaaaagggaataagggcgacacggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcagggttattgtctc
atgagcggatacatatttgaatgtatttagaaaaataaacaaataggggttccgcgcacatttccccgaaaagtgccac (SEQ ID NO:142)

The two PCR products were subjected to fusion PCR as known in the art to create the 1.5 kb fusion. The resulting fusion product was then cloned into PCR2.1TOPO to produce pCP609 (*See*, Figure 16) and sequence below).

caggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctattacgccagctggcgaaagggggatgtgctgcaa
ggcgattaagttgggtaacgccagggttttcccagtcacgacgttgtaaaacgacggccagtgagcgcgcgtaatacgactcact
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ggacatagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccgcttcctcgtgctttacggtatcgccg
ctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgaattgaaaaaggaagagtatgagtattcaacatttcc
gtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatc
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cctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatg

gaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcg
tgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggca
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agttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgc
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gagcgcagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcg
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gccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcccaatacgcaaaccgcctctccccgcgcgtt
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cactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaa
acagctatgaccatgattacgccaagcttggtaccgagctcggatccactagtaacggccgccagtgtgctggaattcgccctt
(SEQ ID NO:143)

The plasmid PCP609 was digested with *Bam*H1/*Xma*I to release the fragment containing the pAprE-*aprE*-stop-pSpoVG-*phd* construct and ligated into pBSFNASally digested with *XmaI*/*BclI* to give the plasmid pCP649. Figure 17 provides a map of pCP649. The complete sequence of pCP649 is provided below.

tagaactagtggatcccccgggctgcaggaattctccatttcttctgctatcaaaataacagactcgtgattttccaaacgagctttc
aaaaaagcctctgccccttgcaaatcggatgcctgtctataaaattcccgatattggttaaacagcggcgcaatggcggccgcatc
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gcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaacc
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gttaccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcctgactccccgtcgtgtagataa
ctacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctccagatttatcagc
aataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaattgttgccgg
gaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgtcgtttg
gtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcg
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ccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatagtgtatgcggcgaccgagttgctcttgcccggc
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ggatcttaccgctgttgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctg
ggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttcctttt
tcaatattattgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaataggggttccg
cgcacatttccccgaaaagtgccacctaaattgtaagcgttaatattttgttaaaattcgcgttaaatttttgttaaatcagctcattttttta
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agtccactattaaagaacgtggactccaacgtcaaagggcgaaaaaccgtctatcagggcgatggcccactacgtgaaccatca
ccctaatcaagttttttggggtcgaggtgccgtaaagcactaaatcggaaccctaaagggagcccccgatttagagcttgacggg
gaaagccggcgaacgtggcgagaaaggaagggaagaaagcgaaaggagcgggcgctagggcgctggcaagtgtagcggt
cacgctgcgcgtaaccaccacacccgccgcgcttaatgcgccgctacagggcgcgtcccattcgccattcaggctgcgcaactg
ttgggaagggcgatcggtgcgggcctcttcgctattacgccagctggcgaaagggggatgtgctgcaaggcgattaagttgggt
aacgccagggttttcccagtcacgacgttgtaaaacgacggccagtgagcgcgcgtaatacgactcactatagggcgaattgga
gctccaccgcggtggcggccgctc (SEQ ID NO:144)

All constructs were confirmed by sequence analysis. PCR reactions were done using Hercules polymerase (Roche) as per the manufacturer's directions.

pCP649 was transformed into *B. subtilis comK pnbA* and integrants selected on L agar containing chloramphenicol (5μg/ml). The activity of the expressed perhydrolase was determined by the pNB activity assay as described herein. The results indicated that the perhydrolase was expressed and active

EXAMPLE 7
Expression of the Perhydrolase in *Streptomyces*.

In this Example, experiments conducted to assess the expression of the perhydrolase in *Streptomyces* are described. To test expression of the perhydrolase in *Streptomyces*, a replicating plasmid was constructed with the *phd* gene being expressed from either the glucose isomerase (GIT) or the A4 promoter (*See e.g.*, US/PCT____/______, filed November 18, 2004, herein incorporated by reference). However, it is not intended that the present invention be limited to these specific promoters, as any suitable promoter will find use with the present invention. Also, although the strain used for perhydrolase expression in this Example was *Streptomyces lividans* TK-23, it is contemplated that any *Streptomyces* will find use in the present invention.

The *Streptomyces* strains were transformed and manipulated using methods known in the art (*See e.g.*, Kieser *et al.*, Practical Streptomyces Genetics, John Innes [2000]).

Construction of pSECGT-MSAT and pSECA4-MSAT

Using standard methods known in the art, the *phd* coding sequence (*See*, Example 4) was cloned into pSECGT to place the gene under control of the GI promoter. Similarly, the gene was cloned in the same plasmid with the A4 promoter using methods known in the art (*See e.g.*, US/PCT____/______, filed November 18, 2004, herein incorporated by reference). Transformants were first selected in *E. coli*, verified by sequence analysis, and then transformed into *S. lividans* TK-23 using methods known in the art (*See e.g.*, Kieser *et al.*, [2000], *supra*). The correct clones expressed from the GI promoter and the A4 promoter were designated "pSECGT-MSAT" and "pSECA4-phd." The sequence of pSECGT-MSAT is provided below, while Figure 18 provides a map of the plasmid.

ctagagtcgaccacgcaggccgccaggtagtcgacgttgatctcgcagccgagcccggccggaccggcggcgctgagcgcg
aggccgacggcgggacggccggcaccggtacgcggtggcgggtcgagttcggtgagcagcccaccggcgatcaggtcgtcg

acgagcgcggagacggtggcccgggtgagcccggtgacggcggcaactcccgcgcgggagagccgatctgtgctgtttgcc
acggtatgcagcaccagcgcgagattatgggctcgcacgctcgactgtcggacgggggcactggaacgagaagtcaggcgag
ccgtcacgcccttgacaatgccacatcctgagcaaataattcaaccactaaacaaatcaaccgcgtttcccggaggtaaccatggc
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cgacgtgcgctggaccggtgtgctggcccagcagctcggagcggacttcgaggtgatcgaggagggactgagcgcgcgcac
caccaacatcgacgaccccaccgatccgcggctcaacggcgcgagctacctgccgtcgtgcctcgcgacgcacctgccgctcg
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tgctcgtcacgcaggtgctcaccagcgcgggcggcgtcggcaccacgtacccggcacccaaggtgctggtggtctcgccgcca
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gccgccttgatgacgtagggaaagttgtaccgcaaaaaacgcagcctgaactagttgcgatcct (SEQ ID NO:145)

Figure 19 provides a map of pSEGT-phdA4, while the sequence is provided below:

ctagagatcgaacttcatgttcgagttcttgttcacgtagaagccggagatgtgagaggtgatctggaactgctcaccctcgttggt
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gcggccgggccggagcggggcgaagacaggagcgtcggccgggccgtgggccgggccgcgcggcccgctcgcgggccg
ccttgatgacgtagggaaagttgtaccgcaaaaaacgcagcctgaactagttgcgatcct (SEQ ID NO:146)

Two colonies of *S. lividans* TK-23 pSECA4-phd were inoculated in 10 ml of TS medium + 50 ppm thiostrepton and incubated at 37°C with shaking at 200 rpm for 2 days. Three mls of broth were used to inoculate 50 ml of Streptomyces Production medium 1 and the culture was incubated for 4 days at 37°C with shaking at 200 rpm.

A sample was taken to assay perhydrolase activity measurement as follows: 10 µls of 20 mg/ml lysozyme were added to 200 µl of sample. After 1 hour of incubation at 37°C, samples were centrifuged and activity was measured using the pNB activity assay described herein. SDS-PAGE and Western blots were also prepared using both clones (pSECA4-phd and pSECGT-MSAT), as known in the art. Briefly, after SDS-PAGE, the proteins were transferred to PVDF membrane and Western blot analysis was conducted. The perhydrolase was detected using an anti-perhydrolase polyclonal anti-sera (1:500 dilution) prepared against purified perhydrolase protein by Covance. The blot was developed using the ECL kit from Amersham. The results indicated that *Streptomyces lividans* strains were capable of expressing active perhydrolase.

EXAMPLE 8

Site-Scanning Mutagenesis of the *M. smegmatis* Perhydrolase Gene

In this Example, experiments involving site-scanning mutagenesis of the *M. smegmatis* perhydrolase gene are described. In these experiments, the QuikChange® site-directed mutagenesis (QC; Stratagene) kit or the QuikChange® Multi Site-Directed mutagenesis (QCMS; Stratagene) kit was used to create site-saturation libraries at each codon in the entire *M. smegmatis* perhydrolase gene contained in the pMSAT-NcoI plasmid. Each perhydrolase codon was mutagenized by replacement with the NNG/C (NNS; 32 combinations) degenerate codon, which encodes for all 20 amino acids and one stop codon. In the case of the QC method, complementary overlapping primers were designed for each codon of interest with 18 bases flanking the NNS codon (*See*, Tables 8-1 and 8-2). A comparison of cartridge purified versus unpurified primers (desalted only) revealed a better representation of amino acids in the libraries made with purified primers (15-19 amino acids versus 11-16 with unpurified primers). Thus, a majority of the libraries were created with the QC method and purified primers. A small number of the libraries were made using the QCMS method and a single 5' phosphorylated forward primer containing 18 bases flanking both sides of the NNS codon (*See*, Table 8-1), however this method resulted in a greater wild type background and fewer amino acid substitutions per site compared to the QC methods. Libraries "nsa301" and "nsa302" were made using the QCMS method, but a trinucleotide mix made up of a single codon for each of the 20 amino acids (*i.e.*, rather than 32 possibilities encoded by NNS for the 20 amino acids) was incorporated within the primers at the sites of interest.

Table 8-1. Site-Saturation Forward Primers

Residue	Primer	Primer Sequence
M1	nsa202F	taacaggaggaattaaccnnsgccaagcgaattctgtgt (SEQ ID NO:147)
A2	nsa203F	caggaggaattaaccatgnnsaagcgaattctgtgtttc (SEQ ID NO:148)

K3	nsa204F	gaggaattaaccatggccnnscgaattctgtgtttcggt (SEQ ID NO:149)
R4	nsa205F	gaattaaccatggccaagnnsattctgtgtttcggtgat (SEQ ID NO:150)
I5	nsa206F	ttaaccatggccaagcgannsctgtgtttcggtgattcc (SEQ ID NO:151)
L6	nsa207F	accatggccaagcgaattnnstgtttcggtgattccctg (SEQ ID NO:152)
C7	nsa208F	atggccaagcgaattctgnnsttcggtgattccctgacc (SEQ ID NO:153)
F8	nsa209F	gccaagcgaattctgtgtnnsggtgattccctgacctgg (SEQ ID NO:154)
G9	nsa210F	aagcgaattctgtgtttcnnsgattccctgacctggggc (SEQ ID NO:155)
D10	nsa168F	cgaattctgtgtttcggtnnstccctgacctggggctgg (SEQ ID NO:156)
S11	nsa212F	attctgtgtttcggtgatnnsctgacctggggctgggtc (SEQ ID NO:157)
L12	nsa169F	ctgtgtttcggtgattccnnsacctggggctgggtcccc (SEQ ID NO:158)
T13	nsa170F	tgtttcggtgattccctgnnstggggctgggtccccgtc (SEQ ID NO:159)
W14	nsa171F	ttcggtgattccctgaccnnsggctgggtccccgtcgaa (SEQ ID NO:160)
G15	nsa216F	ggtgattccctgacctggnnstgggtccccgtcgaagac (SEQ ID NO:161)
W16	nsa172F	gattccctgacctggggcnnsgtccccgtcgaagacggg (SEQ ID NO:162)
V17	nsa218F	tccctgacctggggctggnnscccgtcgaagacggggca (SEQ ID NO:163)
P18	nsa219F	ctgacctggggctgggtcnnsgtcgaagacggggcaccc (SEQ ID NO:164)
V19	nsa220F	acctggggctgggtccccnnsgaagacggggcacccacc (SEQ ID NO:165)
E20	nsa221F	tggggctgggtccccgtcnnsgacggggcacccaccgag (SEQ ID NO:166)
D21	nsa222F	ggctgggtccccgtcgaannsggggcacccaccgagcgg (SEQ ID NO:167)
G22	nsa223F	tgggtccccgtcgaagacnnsgcacccaccgagcggttc (SEQ ID NO:168)
A23	nsa224F	gtccccgtcgaagacgggnnscccaccgagcggttcgcc (SEQ ID NO:169)
P24	nsa191F	cccgtcgaagacggggcannsaccgagcggttcgccccc (SEQ ID NO:170)
T25	nsa192F	gtcgaagacggggcacccnnsgagcggttcgcccccgac (SEQ ID NO:171)
E26	nsa227F	gaagacggggcacccaccnnscggttcgcccccgacgtg (SEQ ID NO:172)
R27	nsa228F	gacggggcacccaccgagnnsttcgcccccgacgtgcgc (SEQ ID NO:173)
F28	nsa229F	ggggcacccaccgagcggnnsgcccccgacgtgcgctgg (SEQ ID NO:174)
A29	nsa230F	gcacccaccgagcggttcnnscccgacgtgcgctggacc (SEQ ID NO:175)
P30	nsa231F	cccaccgagcggttcgccnnsgacgtgcgctggaccggt (SEQ ID NO:176)
D31	nsa232F	accgagcggttcgcccccnnsgtgcgctggaccggtgtg (SEQ ID NO:177)
V32	nsa233F	gagcggttcgcccccgacnnscgctggaccggtgtgctg (SEQ ID NO:178)
R33	nsa234F	cggttcgcccccgacgtgnnstggaccggtgtgctggcc (SEQ ID NO:179)
W34	nsa235F	ttcgcccccgacgtgcgcnnsaccggtgtgctggcccag (SEQ ID NO:180)
T35	nsa236F	gcccccgacgtgcgctggnnsggtgtgctggcccagcag (SEQ ID NO:181)

G36	nsa237F	cccgacgtgcgctggaccnnsgtgctggcccagcagctc (SEQ ID NO:182)
V37	nsa238F	gacgtgcgctggaccggtnnsctggcccagcagctcgga (SEQ ID NO:183)
L38	nsa239F	gtgcgctggaccggtgtgnnsgcccagcagctcggagcg (SEQ ID NO:184)
A39	nsa240F	cgctggaccggtgtgctgnnscagcagctcggagcggac (SEQ ID NO:185)
Q40	nsa241F	tggaccggtgtgctggccnnscagctcggagcggacttc (SEQ ID NO:186)
Q41	nsa242F	accggtgtgctggcccagnnsctcggagcggacttcgag (SEQ ID NO:187)
L42	nsa243F	ggtgtgctggcccagcagnnsggagcggacttcgaggtg (SEQ ID NO:188)
G43	nsa244F	gtgctggcccagcagctcnnsgcggacttcgaggtgatc (SEQ ID NO:189)
A44	nsa245F	ctggcccagcagctcggannsgacttcgaggtgatcgag (SEQ ID NO:190)
D45	nsa246F	gcccagcagctcggagcgnnsttcgaggtgatcgaggag (SEQ ID NO:191)
F46	nsa247F	cagcagctcggagcggacnnsgaggtgatcgaggaggga (SEQ ID NO:192)
E47	nsa248F	cagctcggagcggacttcnnsgtgatcgaggagggactg (SEQ ID NO:193)
V48	nsa249F	ctcggagcggacttcgagnnsatcgaggagggactgagc (SEQ ID NO:194)
I49	nsa250F	ggagcggacttcgaggtgnnsgaggagggactgagcgcg (SEQ ID NO:195)
E50	nsa251F	gcggacttcgaggtgatcnnsgagggactgagcgcgcgc (SEQ ID NO:196)
E51	nsa252F	gacttcgaggtgatcgagnnsggactgagcgcgcgcacc (SEQ ID NO:197)
G52	nsa253F	ttcgaggtgatcgaggagnnsctgagcgcgcgcaccacc (SEQ ID NO:198)
L53	nsa193F	gaggtgatcgaggagggannsagcgcgcgcaccaccaac (SEQ ID NO:199)
S54	nsa173F	gtgatcgaggagggactgnnsgcgcgcaccaccaacatc (SEQ ID NO:200)
A55	nsa174F	atcgaggagggactgagcnnscgcaccaccaacatcgac (SEQ ID NO:201)
R56	nsa257F	gaggagggactgagcgcgnnsaccaccaacatcgacgac (SEQ ID NO:202)
T57	nsa258F	gagggactgagcgcgcgcnnsaccaacatcgacgacccc (SEQ ID NO:203)
T58	nsa259F	ggactgagcgcgcgcaccnnsaacatcgacgaccccacc (SEQ ID NO:204)
N59	nsa260F	ctgagcgcgcgcaccaccnnsatcgacgaccccaccgat (SEQ ID NO:205)
I60	nsa261F	agcgcgcgcaccaccaacnnsgacgaccccaccgatccg (SEQ ID NO:206)
D61	nsa262F	gcgcgcaccaccaacatcnnsgaccccaccgatccgcgg (SEQ ID NO:207)
D62	nsa263F	cgcaccaccaacatcgacnnscccaccgatccgcggctc (SEQ ID NO:208)
P63	nsa264F	accaccaacatcgacgacnnsaccgatccgcggctcaac (SEQ ID NO:209)
T64	nsa194F	accaacatcgacgaccccnnsgatccgcggctcaacggc (SEQ ID NO:210)
D65	nsa195F	aacatcgacgaccccaccnnsccgcggctcaacggcgcg (SEQ ID NO:211)
P66	nsa267F	atcgacgaccccaccgatnnscggctcaacggcgcgagc (SEQ ID NO:212)
R67	nsa196F	gacgaccccaccgatccgnnsctcaacggcgcgagctac (SEQ ID NO:213)
L68	nsa269F	gaccccaccgatccgcggnnsaacggcgcgagctacctg (SEQ ID NO:214)

N69	nsa270F	cccaccgatccgcggctcnnsggcgcgagctacctgccg (SEQ ID NO:215)
G70	nsa271F	accgatccgcggctcaacnnsgcgagctacctgccgtcg (SEQ ID NO:216)
A71	nsa272F	gatccgcggctcaacggcnnsagctacctgccgtcgtgc (SEQ ID NO:217)
S72	nsa273F	ccgcggctcaacggcgcgnnstacctgccgtcgtgcctc (SEQ ID NO:218)
Y73	nsa274F	cggctcaacggcgcgagcnnsctgccgtcgtgcctcgcg (SEQ ID NO:219)
L74	nsa275F	ctcaacggcgcgagctacnnsccgtcgtgcctcgcgacg (SEQ ID NO:220)
P75	nsa276F	aacggcgcgagctacctgnnstcgtgcctcgcgacgcac (SEQ ID NO:221)
S76	nsa277F	ggcgcgagctacctgccgnnstgcctcgcgacgcacctg (SEQ ID NO:222)
C77	nsa278F	gcgagctacctgccgtcgnnsctcgcgacgcacctgccg (SEQ ID NO:223)
L78	nsa279F	agctacctgccgtcgtgcnnsgcgacgcacctgccgctc (SEQ ID NO:224)
A79	nsa280F	tacctgccgtcgtgcctcnnsacgcacctgccgctcgac (SEQ ID NO:225)
T80	nsa281F	ctgccgtcgtgcctcgcgnnscacctgccgctcgacctg (SEQ ID NO:226)
H81	nsa282F	ccgtcgtgcctcgcgacgnnsctgccgctcgacctggtg (SEQ ID NO:227)
L82	nsa283F	tcgtgcctcgcgacgcacnnsccgctcgacctggtgatc (SEQ ID NO:228)
P83	nsa284F	tgcctcgcgacgcacctgnnsctcgacctggtgatcatc (SEQ ID NO:229)
L84	nsa285F	ctcgcgacgcacctgccgnnsgacctggtgatcatcatg (SEQ ID NO:230)
D85	nsa286F	gcgacgcacctgccgctcnnsctggtgatcatcatgctg (SEQ ID NO:231)
L86	nsa287F	acgcacctgccgctcgacnnsgtgatcatcatgctgggc (SEQ ID NO:232)
V87	nsa288F	cacctgccgctcgacctgnnsatcatcatgctgggcacc (SEQ ID NO:233)
I88	nsa289F	ctgccgctcgacctggtgnnsatcatgctgggcaccaac (SEQ ID NO:234)
I89	nsa290F	ccgctcgacctggtgatcnnsatgctgggcaccaacgac (SEQ ID NO:235)
M90	nsa291F	ctcgacctggtgatcatcnnsctgggcaccaacgacacc (SEQ ID NO:236)
L91	nsa292F	gacctggtgatcatcatgnnsggcaccaacgacaccaag (SEQ ID NO:237)
G92	nsa293F	ctggtgatcatcatgctgnnsaccaacgacaccaaggcc (SEQ ID NO:238)
T93	nsa294F	gtgatcatcatgctgggcnnsaacgacaccaaggcctac (SEQ ID NO:239)
N94	nsa175F	atcatcatgctgggcaccnnsgacaccaaggcctacttc (SEQ ID NO:240)
D95	nsa197F	atcatgctgggcaccaacnnsaccaaggcctacttccgg (SEQ ID NO:241)
T96	nsa297F	atgctgggcaccaacgacnnsaaggcctacttccggcgc (SEQ ID NO:242)
K97	nsa176F	ctgggcaccaacgacaccnnsgcctacttccggcgcacc (SEQ ID NO:243)
A98	nsa299F	ggcaccaacgacaccaagnnstacttccggcgcaccccg (SEQ ID NO:244)
Y99	nsa177F	accaacgacaccaaggccnnsttccggcgcaccccgctc (SEQ ID NO:245)
F100	nsa301F	aacgacaccaaggcctacXXXcggcgcaccccgctcgac (SEQ ID NO:246)
R101	nsa302F	gacaccaaggcctacttcXXXcgcaccccgctcgacatc (SEQ ID NO:247)

R102	nsa303F	accaaggcctacttccggnnsaccccgctcgacatcgcg (SEQ ID NO:248)
T103	nsa304F	aaggcctacttccggcgcnnsccgctcgacatcgcgctg (SEQ ID NO:249)
P104	nsa305F	gcctacttccggcgcaccnnsctcgacatcgcgctgggc (SEQ ID NO:250)
L105	nsa306F	tacttccggcgcaccccgnnsgacatcgcgctgggcatg (SEQ ID NO:251)
D106	nsa307F	ttccggcgcaccccgctcnnsatcgcgctgggcatgtcg (SEQ ID NO:252)
I107	nsa308F	cggcgcaccccgctcgacnnsgcgctgggcatgtcggtg (SEQ ID NO:253)
A108	nsa309F	cgcaccccgctcgacatcnnsctgggcatgtcggtgctc (SEQ ID NO:254)
L109	nsa310F	accccgctcgacatcgcgnnsggcatgtcggtgctcgtc (SEQ ID NO:255)
G110	nsa311F	ccgctcgacatcgcgctgnnsatgtcggtgctcgtcacg (SEQ ID NO:256)
M111	nsa312F	ctcgacatcgcgctgggcnnstcggtgctcgtcacgcag (SEQ ID NO:257)
S112	nsa313F	gacatcgcgctgggcatgnnsgtgctcgtcacgcaggtg (SEQ ID NO:258)
V113	nsa314F	atcgcgctgggcatgtcgnnsctcgtcacgcaggtgctc (SEQ ID NO:259)
L114	nsa315F	gcgctgggcatgtcggtgnnsgtcacgcaggtgctcacc (SEQ ID NO:260)
V115	nsa316F	ctgggcatgtcggtgctcnnsacgcaggtgctcaccagc (SEQ ID NO:261)
T116	nsa317F	ggcatgtcggtgctcgtcnnscaggtgctcaccagcgcg (SEQ ID NO:262)
Q117	nsa318F	atgtcggtgctcgtcacgnnsgtgctcaccagcgcgggc (SEQ ID NO:263)
V118	nsa319F	tcggtgctcgtcacgcagnnsctcaccagcgcgggcggc (SEQ ID NO:264)
L119	nsa320F	gtgctcgtcacgcaggtgnnsaccagcgcgggcggcgtc (SEQ ID NO:265)
T120	nsa321F	ctcgtcacgcaggtgctcnnsagcgcgggcggcgtcggc (SEQ ID NO:266)
S121	nsa322F	gtcacgcaggtgctcaccnnsgcgggcggcgtcggcacc (SEQ ID NO:267)
A122	nsa323F	acgcaggtgctcaccagcnnsggcggcgtcggcaccacg (SEQ ID NO:268)
G123	nsa324F	caggtgctcaccagcgcgnnsggcgtcggcaccacgtac (SEQ ID NO:269)
G124	nsa325F	gtgctcaccagcgcgggcnnsgtcggcaccacgtacccg (SEQ ID NO:270)
V125	nsa198F	ctcaccagcgcgggcggcnnsggcaccacgtacccggca (SEQ ID NO:271)
G126	nsa327F	accagcgcgggcggcgtcnnsaccacgtacccggcaccc (SEQ ID NO:272)
T127	nsa328F	agcgcgggcggcgtcggcnnsacgtacccggcacccaag (SEQ ID NO:273)
T128	nsa329F	gcgggcggcgtcggcaccnnstacccggcacccaaggtg (SEQ ID NO:274)
Y129	nsa330F	ggcggcgtcggcaccacgnnsccggcacccaaggtgctg (SEQ ID NO:275)
P130	nsa331F	ggcgtcggcaccacgtacnnsgcacccaaggtgctggtg (SEQ ID NO:276)
A131	nsa332F	gtcggcaccacgtacccgnnscccaaggtgctggtggtc (SEQ ID NO:277)
P132	nsa333F	ggcaccacgtacccggcannsaaggtgctggtggtctcg (SEQ ID NO:278)
K133	nsa334F	accacgtacccggcacccnnsgtgctggtggtctcgccg (SEQ ID NO:279)
V134	nsa335F	acgtacccggcacccaagnnsctggtggtctcgccgcca (SEQ ID NO:280)

L135	nsa336F	taccccggcacccaaggtgnnsgtggtctcgccgccaccg (SEQ ID NO:281)
V136	nsa337F	ccggcacccaaggtgctgnnsgtctcgccgccaccgctg (SEQ ID NO:282)
V137	nsa338F	gcacccaaggtgctggtgnnstcgccgccaccgctggcg (SEQ ID NO:283)
S138	nsa339F	cccaaggtgctggtggtcnnsccgccaccgctggcgccc (SEQ ID NO:284)
P139	nsa340F	aaggtgctggtggtctcgnnsccaccgctggcgcccatg (SEQ ID NO:285)
P140	nsa341F	gtgctggtggtctcgccgnnsccgctggcgcccatgccg (SEQ ID NO:286)
P141	nsa342F	ctggtggtctcgccgccannsctggcgcccatgccgcac (SEQ ID NO:287)
L142	nsa343F	gtggtctcgccgccaccgnnsgcgcccatgccgcacccc (SEQ ID NO:288)
A143	nsa344F	gtctcgccgccaccgctgnnscccatgccgcacccctgg (SEQ ID NO:289)
P144	nsa345F	tcgccgccaccgctggcgnnsatgccgcacccctggttc (SEQ ID NO:290)
M145	nsa346F	ccgccaccgctggcgcccnnsccgcacccctggttccag (SEQ ID NO:291)
P146	nsa178F	ccaccgctggcgcccatgnnscacccctggttccagttg (SEQ ID NO:292)
H147	nsa348F	ccgctggcgcccatgccgnnsccctggttccagttgatc (SEQ ID NO:293)
P148	nsa199F	ctggcgcccatgccgcacnnstggttccagttgatcttc (SEQ ID NO:294)
W149	nsa179F	gcgcccatgccgcaccccnnsttccagttgatcttcgag (SEQ ID NO:295)
F150	nsa180F	cccatgccgcacccctggnnscagttgatcttcgagggc (SEQ ID NO:296)
Q151	nsa352F	atgccgcacccctggttcnnsttgatcttcgagggcggc (SEQ ID NO:297)
L152	nsa353F	ccgcacccctggttccagnnsatcttcgagggcggcgag (SEQ ID NO:298)
I153	nsa200F	cacccctggttccagttgnnsttcgagggcggcgagcag (SEQ ID NO:299)
F154	nsa201F	ccctggttccagttgatcnnsgagggcggcgagcagaag (SEQ ID NO:300)
E155	nsa356F	tggttccagttgatcttcnnsggcggcgagcagaagacc (SEQ ID NO:301)
G156	nsa357F	ttccagttgatcttcgagnnsggcgagcagaagaccact (SEQ ID NO:302)
G157	nsa358F	cagttgatcttcgagggcnnsgagcagaagaccactgag (SEQ ID NO:303)
E158	nsa359F	ttgatcttcgagggcggcnnscagaagaccactgagctc (SEQ ID NO:304)
Q159	nsa360F	atcttcgagggcggcgagnnsaagaccactgagctcgcc (SEQ ID NO:305)
K160	nsa361F	ttcgagggcggcgagcagnnsaccactgagctcgcccgc (SEQ ID NO:306)
T161	nsa362F	gagggcggcgagcagaagnnsactgagctcgcccgcgtg (SEQ ID NO:307)
T162	nsa363F	ggcggcgagcagaagaccnnsgagctcgcccgcgtgtac (SEQ ID NO:308)
E163	nsa364F	ggcgagcagaagaccactnnsctcgcccgcgtgtacagc (SEQ ID NO:309)
L164	nsa365F	gagcagaagaccactgagnnsgcccgcgtgtacagcgcg (SEQ ID NO:310)
A165	nsa366F	cagaagaccactgagctcnnscgcgtgtacagcgcgctc (SEQ ID NO:311)
R166	nsa367F	aagaccactgagctcgccnnsgtgtacagcgcgctcgcg (SEQ ID NO:312)
V167	nsa368F	accactgagctcgcccgcnnstacagcgcgctcgcgtcg (SEQ ID NO:313)

Y168	nsa369F	actgagctcgcccgcgtgnnsagcgcgctcgcgtcgttc (SEQ ID NO:314)
S169	nsa370F	gagctcgcccgcgtgtacnnsgcgctcgcgtcgttcatg (SEQ ID NO:315)
A170	nsa371F	ctcgcccgcgtgtacagcnnsctcgcgtcgttcatgaag (SEQ ID NO:316)
L171	nsa372F	gcccgcgtgtacagcgcgnnsgcgtcgttcatgaaggtg (SEQ ID NO:317)
A172	nsa373F	cgcgtgtacagcgcgctcnnstcgttcatgaaggtgccg (SEQ ID NO:318)
S173	nsa374F	gtgtacagcgcgctcgcgnnsttcatgaaggtgccgttc (SEQ ID NO:319)
F174	nsa375F	tacagcgcgctcgcgtcgnnsatgaaggtgccgttcttc (SEQ ID NO:320)
M175	nsa376F	agcgcgctcgcgtcgttcnnsaaggtgccgttcttcgac (SEQ ID NO:321)
K176	nsa377F	gcgctcgcgtcgttcatgnnsgtgccgttcttcgacgcg (SEQ ID NO:322)
V177	nsa378F	ctcgcgtcgttcatgaagnnsccgttcttcgacgcgggt (SEQ ID NO:323)
P178	nsa379F	gcgtcgttcatgaaggtgnnsttcttcgacgcgggttcg (SEQ ID NO:324)
F179	nsa380F	tcgttcatgaaggtgccgnnsttcgacgcgggttcggtg (SEQ ID NO:325)
F180	nsa381F	ttcatgaaggtgccgttcnnsgacgcgggttcggtgatc (SEQ ID NO:326)
D181	nsa382F	atgaaggtgccgttcttcnnsgcgggttcggtgatcagc (SEQ ID NO:327)
A182	nsa383F	aaggtgccgttcttcgacnnsggttcggtgatcagcacc (SEQ ID NO:328)
G183	nsa384F	gtgccgttcttcgacgcgnnstcggtgatcagcaccgac (SEQ ID NO:329)
S184	nsa385F	ccgttcttcgacgcgggtnnsgtgatcagcaccgacggc (SEQ ID NO:330)
V185	nsa386F	ttcttcgacgcgggttcgnnsatcagcaccgacggcgtc (SEQ ID NO:331)
I186	nsa387F	ttcgacgcgggttcggtgnnsagcaccgacggcgtcgac (SEQ ID NO:332)
S187	nsa388F	gacgcgggttcggtgatcnnsaccgacggcgtcgacgga (SEQ ID NO:333)
T188	nsa389F	gcgggttcggtgatcagcnnsgacggcgtcgacggaatc (SEQ ID NO:334)
D189	nsa390F	ggttcggtgatcagcaccnnsggcgtcgacggaatccac (SEQ ID NO:335)
G190	nsa391F	tcggtgatcagcaccgacnnsgtcgacggaatccacttc (SEQ ID NO:336)
V191	nsa392F	gtgatcagcaccgacggcnnsgacggaatccacttcacc (SEQ ID NO:337)
D192	nsa393F	atcagcaccgacggcgtcnnsggaatccacttcaccgag (SEQ ID NO:338)
G193	nsa394F	agcaccgacggcgtcgacnnsatccacttcaccgaggcc (SEQ ID NO:339)
I194	nsa181F	accgacggcgtcgacggannscacttcaccgaggccaac (SEQ ID NO:340)
H195	nsa396F	gacggcgtcgacggaatcnnsttcaccgaggccaacaat (SEQ ID NO:341)
F196	nsa182F	ggcgtcgacggaatccacnnsaccgaggccaacaatcgc (SEQ ID NO:342)
T197	nsa398F	gtcgacggaatccacttcnnsgaggccaacaatcgcgat (SEQ ID NO:343)
E198	nsa399F	gacggaatccacttcaccnnsgccaacaatcgcgatctc (SEQ ID NO:344)
A199	nsa400F	ggaatccacttcaccgagnnsaacaatcgcgatctcggg (SEQ ID NO:345)
N200	nsa401F	atccacttcaccgaggccnnsaatcgcgatctcggggtg (SEQ ID NO:346)

N201	nsa402F	cacttcaccgaggccaacnnscgcgatctcggggtggcc (SEQ ID NO:347)
R202	nsa403F	ttcaccgaggccaacaatnnsgatctcggggtggccctc (SEQ ID NO:348)
D203	nsa404F	accgaggccaacaatcgcnnsctcggggtggccctcgcg (SEQ ID NO:349)
L204	nsa405F	gaggccaacaatcgcgatnnsggggtggccctcgcggaa (SEQ ID NO:350)
G205	nsa406F	gccaacaatcgcgatctcnnsgtggccctcgcggaacag (SEQ ID NO:351)
V206	nsa407F	aacaatcgcgatctcgggnnsgccctcgcggaacaggtg (SEQ ID NO:352)
A207	nsa408F	aatcgcgatctcggggtgnnsctcgcggaacaggtgcag (SEQ ID NO:353)
L208	nsa409F	cgcgatctcggggtggccnnsgcggaacaggtgcagagc (SEQ ID NO:354)
A209	nsa410F	gatctcggggtggccctcnnsgaacaggtgcagagcctg (SEQ ID NO:355)
E210	nsa411F	ctcggggtggccctcgcgnnscaggtgcagagcctgctg (SEQ ID NO:356)
Q211	nsa412F	ggggtggccctcgcggaannsgtgcagagcctgctgtaa (SEQ ID NO:357)
V212	nsa413F	gtggccctcgcggaacagnnscagagcctgctgtaaaag (SEQ ID NO:358)
Q213	nsa414F	gccctcgcggaacaggtgnnsagcctgctgtaaaagggc (SEQ ID NO:359)
S214	nsa415F	ctcgcggaacaggtgcagnnsctgctgtaaaagggcgaa (SEQ ID NO:360)
L215	nsa416F	gcggaacaggtgcagagcnnsctgtaaaagggcgaattc (SEQ ID NO:361)
L216	nsa417F	gaacaggtgcagagcctgnnstaaaagggcgaattctgc (SEQ ID NO:362)

Table 8-2 Site-Saturation Reverse Primer Sequences

Residue	Primer	Primer Sequence
M1	nsa202R	ACACAGAATTCGCTTGGCSNNGGTTAATTCCTCCTGTTA (SEQ ID NO:363)
A2	nsa203R	GAAACACAGAATTCGCTTSNNCATGGTTAATTCCTCCTG (SEQ ID NO:364)
K3	nsa204R	ACCGAAACACAGAATTCGSNNGGCCATGGTTAATTCCTC (SEQ ID NO:365)
R4	nsa205R	ATCACCGAAACACAGAATSNNCTTGGCCATGGTTAATTC (SEQ ID NO:366)
I5	nsa206R	GGAATCACCGAAACACAGSNNTCGCTTGGCCATGGTTAA (SEQ ID NO:367)
L6	nsa207R	CAGGGAATCACCGAAACASNNAATTCGCTTGGCCATGGT (SEQ ID NO:368)
C7	nsa208R	GGTCAGGGAATCACCGAASNNCAGAATTCGCTTGGCCAT (SEQ ID NO:369)
F8	nsa209R	CCAGGTCAGGGAATCACCSNNACACAGAATTCGCTTGGC (SEQ ID NO:370)

G9	nsa210R	GCCCCAGGTCAGGGAATCSNNGAAACACAGAATTCGCTT (SEQ ID NO:371)
D10	nsa168R	CCAGCCCCAGGTCAGGGASNNACCGAAACACAGAATTCG (SEQ ID NO:372)
S11	nsa212R	GACCCAGCCCCAGGTCAGSNNATCACCGAAACACAGAAT (SEQ ID NO:373)
L12	nsa169R	GGGGACCCAGCCCCAGGTSNNGGAATCACCGAAACACAG (SEQ ID NO:374)
T13	nsa170R	GACGGGGACCCAGCCCCASNNCAGGGAATCACCGAAACA (SEQ ID NO:375)
W14	nsa171R	TTCGACGGGGACCCAGCCSNNGGTCAGGGAATCACCGAA (SEQ ID NO:376)
G15	nsa216R	GTCTTCGACGGGGACCCASNNCCAGGTCAGGGAATCACC (SEQ ID NO:377)
W16	nsa172R	CCCGTCTTCGACGGGGACSNNGCCCCAGGTCAGGGAATC (SEQ ID NO:378)
V17	nsa218R	TGCCCCGTCTTCGACGGGSNNCCAGCCCCAGGTCAGGGA (SEQ ID NO:379)
P18	nsa219R	GGGTGCCCCGTCTTCGACSNNGACCCAGCCCCAGGTCAG (SEQ ID NO:380)
V19	nsa220R	GGTGGGTGCCCCGTCTTCSNNGGGGACCCAGCCCCAGGT (SEQ ID NO:381)
E20	nsa221R	CTCGGTGGGTGCCCCGTCSNNGACGGGGACCCAGCCCCA (SEQ ID NO:382)
D21	nsa222R	CCGCTCGGTGGGTGCCCCSNNTTCGACGGGGACCCAGCC (SEQ ID NO:383)
G22	nsa223R	GAACCGCTCGGTGGGTGCSNNGTCTTCGACGGGGACCCA (SEQ ID NO:384)
A23	nsa224R	GGCGAACCGCTCGGTGGGSNNCCCGTCTTCGACGGGGAC (SEQ ID NO:385)
P24	nsa191R	GGGGGCGAACCGCTCGGTSNNTGCCCCGTCTTCGACGGG (SEQ ID NO:386)
T25	nsa192R	GTCGGGGGCGAACCGCTCSNNGGGTGCCCCGTCTTCGAC (SEQ ID NO:387)
E26	nsa227R	CACGTCGGGGGCGAACCGSNNGGTGGGTGCCCCGTCTTC (SEQ ID NO:388)
R27	nsa228R	GCGCACGTCGGGGGCGAASNNCTCGGTGGGTGCCCCGTC (SEQ ID NO:389)
F28	nsa229R	CCAGCGCACGTCGGGGGCSNNCCGCTCGGTGGGTGCCCC (SEQ ID NO:390)

A29	nsa230R	GGTCCAGCGCACGTCGGGSNNGAACCGCTCGGTGGGTGC (SEQ ID NO:391)
P30	nsa231R	ACCGGTCCAGCGCACGTCSNNGGCGAACCGCTCGGTGGG (SEQ ID NO:392)
D31	nsa232R	CACACCGGTCCAGCGCACSNNGGGGGCGAACCGCTCGGT (SEQ ID NO:393)
V32	nsa233R	CAGCACACCGGTCCAGCGSNNGTCGGGGGCGAACCGCTC (SEQ ID NO:394)
R33	nsa234R	GGCCAGCACACCGGTCCASNNCACGTCGGGGGCGAACCG (SEQ ID NO:395)
W34	nsa235R	CTGGGCCAGCACACCGGTSNNGCGCACGTCGGGGGCGAA (SEQ ID NO:396)
T35	nsa236R	CTGCTGGGCCAGCACACCSNNCCAGCGCACGTCGGGGGC (SEQ ID NO:397)
G36	nsa237R	GAGCTGCTGGGCCAGCACSNNGGTCCAGCGCACGTCGGG (SEQ ID NO:398)
V37	nsa238R	TCCGAGCTGCTGGGCCAGSNNACCGGTCCAGCGCACGTC (SEQ ID NO:399)
L38	nsa239R	CGCTCCGAGCTGCTGGGCSNNCACACCGGTCCAGCGCAC (SEQ ID NO:400)
A39	nsa240R	GTCCGCTCCGAGCTGCTGSNNCAGCACACCGGTCCAGCG (SEQ ID NO:401)
Q40	nsa241R	GAAGTCCGCTCCGAGCTGSNNGGCCAGCACACCGGTCCA (SEQ ID NO:402)
Q41	nsa242R	CTCGAAGTCCGCTCCGAGSNNCTGGGCCAGCACACCGGT (SEQ ID NO:403)
L42	nsa243R	CACCTCGAAGTCCGCTCCSNNCTGCTGGGCCAGCACACC (SEQ ID NO:404)
G43	nsa244R	GATCACCTCGAAGTCCGCSNNGAGCTGCTGGGCCAGCAC (SEQ ID NO:405)
A44	nsa245R	CTCGATCACCTCGAAGTCSNNTCCGAGCTGCTGGGCCAG (SEQ ID NO:406)
D45	nsa246R	CTCCTCGATCACCTCGAASNNCGCTCCGAGCTGCTGGGC (SEQ ID NO:407)
F46	nsa247R	TCCCTCCTCGATCACCTCSNNGTCCGCTCCGAGCTGCTG (SEQ ID NO:408)
E47	nsa248R	CAGTCCCTCCTCGATCACSNNGAAGTCCGCTCCGAGCTG (SEQ ID NO:409)
V48	nsa249R	GCTCAGTCCCTCCTCGATSNNCTCGAAGTCCGCTCCGAG (SEQ ID NO:410)

I49	nsa250R	CGCGCTCAGTCCCTCCTCSNNCACCTCGAAGTCCGCTCC (SEQ ID NO:411)
E50	nsa251R	GCGCGCGCTCAGTCCCTCSNNGATCACCTCGAAGTCCGC (SEQ ID NO:412)
E51	nsa252R	GGTGCGCGCGCTCAGTCCSNNCTCGATCACCTCGAAGTC (SEQ ID NO:413)
G52	nsa253R	GGTGGTGCGCGCGCTCAGSNNCTCCTCGATCACCTCGAA (SEQ ID NO:414)
L53	nsa193R	GTTGGTGGTGCGCGCGCTSNNTCCCTCCTCGATCACCTC (SEQ ID NO:415)
S54	nsa173R	GATGTTGGTGGTGCGCGCSNNCAGTCCCTCCTCGATCAC (SEQ ID NO:416)
A55	nsa174R	GTCGATGTTGGTGGTGCGSNNGCTCAGTCCCTCCTCGAT (SEQ ID NO:417)
R56	nsa257R	GTCGTCGATGTTGGTGGTSNNCGCGCTCAGTCCCTCCTC (SEQ ID NO:418)
T57	nsa258R	GGGGTCGTCGATGTTGGTSNNGCGCGCGCTCAGTCCCTC (SEQ ID NO:419)
T58	nsa259R	GGTGGGGTCGTCGATGTTSNNGGTGCGCGCGCTCAGTCC (SEQ ID NO:420)
N59	nsa260R	ATCGGTGGGGTCGTCGATSNNGGTGGTGCGCGCGCTCAG (SEQ ID NO:421)
I60	nsa261R	CGGATCGGTGGGGTCGTCSNNGTTGGTGGTGCGCGCGCT (SEQ ID NO:422)
D61	nsa262R	CCGCGGATCGGTGGGGTCSNNGATGTTGGTGGTGCGCGC (SEQ ID NO:423)
D62	nsa263R	GAGCCGCGGATCGGTGGGSNNGTCGATGTTGGTGGTGCG (SEQ ID NO:424)
P63	nsa264R	GTTGAGCCGCGGATCGGTSNNGTCGTCGATGTTGGTGGT (SEQ ID NO:425)
T64	nsa194R	GCCGTTGAGCCGCGGATCSNNGGGGTCGTCGATGTTGGT (SEQ ID NO:426)
D65	nsa195R	CGCGCCGTTGAGCCGCGGSNNGGTGGGGTCGTCGATGTT (SEQ ID NO:427)
P66	nsa267R	GCTCGCGCCGTTGAGCCGSNNATCGGTGGGGTCGTCGAT (SEQ ID NO:428)
R67	nsa196R	GTAGCTCGCGCCGTTGAGSNNCGGATCGGTGGGGTCGTC (SEQ ID NO:429)
L68	nsa269R	CAGGTAGCTCGCGCCGTTSNNCCGCGGATCGGTGGGGTC (SEQ ID NO:430)

N69	nsa270R	CGGCAGGTAGCTCGCGCCSNNGAGCCGCGGATCGGTGGG (SEQ ID NO:431)
G70	nsa271R	CGACGGCAGGTAGCTCGCSNNGTTGAGCCGCGGATCGGT (SEQ ID NO:432)
A71	nsa272R	GCACGACGGCAGGTAGCTSNNGCCGTTGAGCCGCGGATC (SEQ ID NO:433)
S72	nsa273R	GAGGCACGACGGCAGGTASNNCGCGCCGTTGAGCCGCGG (SEQ ID NO:434)
Y73	nsa274R	CGCGAGGCACGACGGCAGSNNGCTCGCGCCGTTGAGCCG (SEQ ID NO:435)
L74	nsa275R	CGTCGCGAGGCACGACGGSNNGTAGCTCGCGCCGTTGAG (SEQ ID NO:436)
P75	nsa276R	GTGCGTCGCGAGGCACGASNNCAGGTAGCTCGCGCCGTT (SEQ ID NO:437)
S76	nsa277R	CAGGTGCGTCGCGAGGCASNNCGGCAGGTAGCTCGCGCC (SEQ ID NO:438)
C77	nsa278R	CGGCAGGTGCGTCGCGAGSNNCGACGGCAGGTAGCTCGC (SEQ ID NO:439)
L78	nsa279R	GAGCGGCAGGTGCGTCGCSNNGCACGACGGCAGGTAGCT (SEQ ID NO:440)
A79	nsa280R	GTCGAGCGGCAGGTGCGTSNNGAGGCACGACGGCAGGTA (SEQ ID NO:441)
T80	nsa281R	CAGGTCGAGCGGCAGGTGSNNCGCGAGGCACGACGGCAG (SEQ ID NO:442)
H81	nsa282R	CACCAGGTCGAGCGGCAGSNNCGTCGCGAGGCACGACGG (SEQ ID NO:443)
L82	nsa283R	GATCACCAGGTCGAGCGGSNNGTGCGTCGCGAGGCACGA (SEQ ID NO:444)
P83	nsa284R	GATGATCACCAGGTCGAGSNNCAGGTGCGTCGCGAGGCA (SEQ ID NO:445)
L84	nsa285R	CATGATGATCACCAGGTCSNNCGGCAGGTGCGTCGCGAG (SEQ ID NO:446)
D85	nsa286R	CAGCATGATGATCACCAGSNNGAGCGGCAGGTGCGTCGC (SEQ ID NO:447)
L86	nsa287R	GCCCAGCATGATGATCACSNNGTCGAGCGGCAGGTGCGT (SEQ ID NO:448)
V87	nsa288R	GGTGCCCAGCATGATGATSNNCAGGTCGAGCGGCAGGTG (SEQ ID NO:449)
I88	nsa289R	GTTGGTGCCCAGCATGATSNNCACCAGGTCGAGCGGCAG (SEQ ID NO:450)

I89	nsa290R	GTCGTTGGTGCCCAGCATSNNGATCACCAGGTCGAGCGG (SEQ ID NO:451)
M90	nsa291R	GGTGTCGTTGGTGCCCAGSNNGATGATCACCAGGTCGAG (SEQ ID NO:452)
L91	nsa292R	CTTGGTGTCGTTGGTGCCSNNCATGATGATCACCAGGTC (SEQ ID NO:453)
G92	nsa293R	GGCCTTGGTGTCGTTGGTSNNCAGCATGATGATCACCAG (SEQ ID NO:454)
T93	nsa294R	GTAGGCCTTGGTGTCGTTSNNGCCCAGCATGATGATCAC (SEQ ID NO:455)
N94	nsa175R	GAAGTAGGCCTTGGTGTCSNNGGTGCCCAGCATGATGAT (SEQ ID NO:456)
D95	nsa197R	CCGGAAGTAGGCCTTGGTSNNGTTGGTGCCCAGCATGAT (SEQ ID NO:457)
T96	nsa297R	GCGCCGGAAGTAGGCCTTSNNGTCGTTGGTGCCCAGCAT (SEQ ID NO:458)
K97	nsa176R	GGTGCGCCGGAAGTAGGCSNNGGTGTCGTTGGTGCCCAG (SEQ ID NO:459)
A98	nsa299R	CGGGGTGCGCCGGAAGTASNNCTTGGTGTCGTTGGTGCC (SEQ ID NO:460)
Y99	nsa177R	GAGCGGGGTGCGCCGGAASNNGGCCTTGGTGTCGTTGGT (SEQ ID NO:461)
F100	nsa301R	GTCGAGCGGGGTGCGCCGSNNGTAGGCCTTGGTGTCGTT (SEQ ID NO:462)
R101	nsa302R	GATGTCGAGCGGGGTGCGSNNGAAGTAGGCCTTGGTGTC (SEQ ID NO:463)
R102	nsa303R	CGCGATGTCGAGCGGGGTSNNCCGGAAGTAGGCCTTGGT (SEQ ID NO:464)
T103	nsa304R	CAGCGCGATGTCGAGCGGSNNGCGCCGGAAGTAGGCCTT (SEQ ID NO:465)
P104	nsa305R	GCCCAGCGCGATGTCGAGSNNGGTGCGCCGGAAGTAGGC (SEQ ID NO:466)
L105	nsa306R	CATGCCCAGCGCGATGTCSNNCGGGGTGCGCCGGAAGTA (SEQ ID NO:467)
D106	nsa307R	CGACATGCCCAGCGCGATSNNGAGCGGGGTGCGCCGGAA (SEQ ID NO:468)
I107	nsa308R	CACCGACATGCCCAGCGCSNNGTCGAGCGGGGTGCGCCG (SEQ ID NO:469)
A108	nsa309R	GAGCACCGACATGCCCAGSNNGATGTCGAGCGGGGTGCG (SEQ ID NO:470)

L109	nsa310R	GACGAGCACCGACATGCCSNNCGCGATGTCGAGCGGGGT (SEQ ID NO:471)
G110	nsa311R	CGTGACGAGCACCGACATSNNCAGCGCGATGTCGAGCGG (SEQ ID NO:472)
M111	nsa312R	CTGCGTGACGAGCACCGASNNGCCCAGCGCGATGTCGAG (SEQ ID NO:473)
S112	nsa313R	CACCTGCGTGACGAGCACSNNCATGCCCAGCGCGATGTC (SEQ ID NO:474)
V113	nsa314R	GAGCACCTGCGTGACGAGSNNCGACATGCCCAGCGCGAT (SEQ ID NO:475)
L114	nsa315R	GGTGAGCACCTGCGTGACSNNCACCGACATGCCCAGCGC (SEQ ID NO:476)
V115	nsa316R	GCTGGTGAGCACCTGCGTSNNGAGCACCGACATGCCCAG (SEQ ID NO:477)
T116	nsa317R	CGCGCTGGTGAGCACCTGSNNGACGAGCACCGACATGCC (SEQ ID NO:478)
Q117	nsa318R	GCCCGCGCTGGTGAGCACSNNCGTGACGAGCACCGACAT (SEQ ID NO:479)
V118	nsa319R	GCCGCCCGCGCTGGTGAGSNNCTGCGTGACGAGCACCGA (SEQ ID NO:480)
L119	nsa320R	GACGCCGCCCGCGCTGGTSNNCACCTGCGTGACGAGCAC (SEQ ID NO:481)
T120	nsa321R	GCCGACGCCGCCCGCGCTSNNGAGCACCTGCGTGACGAG (SEQ ID NO:482)
S121	nsa322R	GGTGCCGACGCCGCCCGCSNNGGTGAGCACCTGCGTGAC (SEQ ID NO:483)
A122	nsa323R	CGTGGTGCCGACGCCGCCSNNGCTGGTGAGCACCTGCGT (SEQ ID NO:484)
G123	nsa324R	GTACGTGGTGCCGACGCCSNNCGCGCTGGTGAGCACCTG (SEQ ID NO:485)
G124	nsa325R	CGGGTACGTGGTGCCGACSNNGCCCGCGCTGGTGAGCAC (SEQ ID NO:486)
V125	nsa198R	TGCCGGGTACGTGGTGCCSNNGCCGCCCGCGCTGGTGAG (SEQ ID NO:487)
G126	nsa327R	GGGTGCCGGGTACGTGGTSNNGACGCCGCCCGCGCTGGT (SEQ ID NO:488)
T127	nsa328R	CTTGGGTGCCGGGTACGTSNNGCCGACGCCGCCCGCGCT (SEQ ID NO:489)
T128	nsa329R	CACCTTGGGTGCCGGGTASNNGGTGCCGACGCCGCCCGC (SEQ ID NO:490)

Y129	msa330R	CAGCACCTTGGGTGCCGGSNNCGTGGTGCCGACGCCGCC (SEQ ID NO:491)
P130	msa331R	CACCAGCACCTTGGGTGCSNNGTACGTGGTGCCGACGCC (SEQ ID NO:492)
A131	msa332R	GACCACCAGCACCTTGGGSNNCGGGTACGTGGTGCCGAC (SEQ ID NO:493)
P132	msa333R	CGAGACCACCAGCACCTTSNNTGCCGGGTACGTGGTGCC (SEQ ID NO:494)
K133	msa334R	CGGCGAGACCACCAGCACSNNGGGTGCCGGGTACGTGGT (SEQ ID NO:495)
V134	msa335R	TGGCGGCGAGACCACCAGSNNCTTGGGTGCCGGGTACGT (SEQ ID NO:496)
L135	msa336R	CGGTGGCGGCGAGACCACSNNCACCTTGGGTGCCGGGTA (SEQ ID NO:497)
V136	msa337R	CAGCGGTGGCGGCGAGACSNNCAGCACCTTGGGTGCCGG (SEQ ID NO:498)
V137	msa338R	CGCCAGCGGTGGCGGCGASNNCACCAGCACCTTGGGTGC (SEQ ID NO:499)
S138	msa339R	GGGCGCCAGCGGTGGCGGSNNGACCACCAGCACCTTGGG (SEQ ID NO:500)
P139	msa340R	CATGGGCGCCAGCGGTGGSNNCGAGACCACCAGCACCTT (SEQ ID NO:501)
P140	msa341R	CGGCATGGGCGCCAGCGGSNNCGGCGAGACCACCAGCAC (SEQ ID NO:502)
P141	msa342R	GTGCGGCATGGGCGCCAGSNNTGGCGGCGAGACCACCAG (SEQ ID NO:503)
L142	msa343R	GGGGTGCGGCATGGGCGCSNNCGGTGGCGGCGAGACCAC (SEQ ID NO:504)
A143	msa344R	CCAGGGGTGCGGCATGGGSNNCAGCGGTGGCGGCGAGAC (SEQ ID NO:505)
P144	msa345R	GAACCAGGGGTGCGGCATSNNCGCCAGCGGTGGCGGCGA (SEQ ID NO:506)
M145	msa346R	CTGGAACCAGGGGTGCGGSNNGGGCGCCAGCGGTGGCGG (SEQ ID NO:507)
P146	msa178R	CAACTGGAACCAGGGGTGSNNCATGGGCGCCAGCGGTGG (SEQ ID NO:508)
H147	msa348R	GATCAACTGGAACCAGGGSNNCGGCATGGGCGCCAGCGG (SEQ ID NO:509)
P148	msa199R	GAAGATCAACTGGAACCASNNGTGCGGCATGGGCGCCAG (SEQ ID NO:510)

W149	nsa179R	CTCGAAGATCAACTGGAASNNGGGGTGCGGCATGGGCGC (SEQ ID NO:511)
F150	nsa180R	GCCCTCGAAGATCAACTGSNNCCAGGGGTGCGGCATGGG (SEQ ID NO:512)
Q151	nsa352R	GCCGCCCTCGAAGATCAASNNGAACCAGGGGTGCGGCAT (SEQ ID NO:513)
L152	nsa353R	CTCGCCGCCCTCGAAGATSNNCTGGAACCAGGGGTGCGG (SEQ ID NO:514)
I153	nsa200R	CTGCTCGCCGCCCTCGAASNNCAACTGGAACCAGGGGTG (SEQ ID NO:515)
F154	nsa201R	CTTCTGCTCGCCGCCCTCSNNGATCAACTGGAACCAGGG (SEQ ID NO:516)
E155	nsa356R	GGTCTTCTGCTCGCCGCCSNNGAAGATCAACTGGAACCA (SEQ ID NO:517)
G156	nsa357R	AGTGGTCTTCTGCTCGCCSNNCTCGAAGATCAACTGGAA (SEQ ID NO:518)
G157	nsa358R	CTCAGTGGTCTTCTGCTCSNNGCCCTCGAAGATCAACTG (SEQ ID NO:519)
E158	nsa359R	GAGCTCAGTGGTCTTCTGSNNGCCGCCCTCGAAGATCAA (SEQ ID NO:520)
Q159	nsa360R	GGCGAGCTCAGTGGTCTTSNNCTCGCCGCCCTCGAAGAT (SEQ ID NO:521)
K160	nsa361R	GCGGGCGAGCTCAGTGGTSNNCTGCTCGCCGCCCTCGAA (SEQ ID NO:522)
T161	nsa362R	CACGCGGGCGAGCTCAGTSNNCTTCTGCTCGCCGCCCTC (SEQ ID NO:523)
T162	nsa363R	GTACACGCGGGCGAGCTCSNNGGTCTTCTGCTCGCCGCC (SEQ ID NO:524)
E163	nsa364R	GCTGTACACGCGGGCGAGSNNAGTGGTCTTCTGCTCGCC (SEQ ID NO:525)
L164	nsa365R	CGCGCTGTACACGCGGGCSNNCTCAGTGGTCTTCTGCTC (SEQ ID NO:526)
A165	nsa366R	GAGCGCGCTGTACACGCGSNNGAGCTCAGTGGTCTTCTG (SEQ ID NO:527)
R166	nsa367R	CGCGAGCGCGCTGTACACSNNGGCGAGCTCAGTGGTCTT (SEQ ID NO:528)
V167	nsa368R	CGACGCGAGCGCGCTGTASNNGCGGGCGAGCTCAGTGGT (SEQ ID NO:529)
Y168	nsa369R	GAACGACGCGAGCGCGCTSNNCACGCGGGCGAGCTCAGT (SEQ ID NO:530)

S169	nsa370R	CATGAACGACGCGAGCGCSNNGTACACGCGGGCGAGCTC (SEQ ID NO:531)
A170	nsa371R	CTTCATGAACGACGCGAGSNNGCTGTACACGCGGGCGAG (SEQ ID NO:532)
L171	nsa372R	CACCTTCATGAACGACGCSNNCGCGCTGTACACGCGGGC (SEQ ID NO:533)
A172	nsa373R	CGGCACCTTCATGAACGASNNGAGCGCGCTGTACACGCG (SEQ ID NO:534)
S173	nsa374R	GAACGGCACCTTCATGAASNNCGCGAGCGCGCTGTACAC (SEQ ID NO:535)
F174	nsa375R	GAAGAACGGCACCTTCATSNNCGACGCGAGCGCGCTGTA (SEQ ID NO:536)
M175	nsa376R	GTCGAAGAACGGCACCTTSNNGAACGACGCGAGCGCGCT (SEQ ID NO:537)
K176	nsa377R	CGCGTCGAAGAACGGCACSNNCATGAACGACGCGAGCGC (SEQ ID NO:538)
V177	nsa378R	ACCCGCGTCGAAGAACGGSNNCTTCATGAACGACGCGAG (SEQ ID NO:539)
P178	nsa379R	CGAACCCGCGTCGAAGAASNNCACCTTCATGAACGACGC (SEQ ID NO:540)
F179	nsa380R	CACCGAACCCGCGTCGAASNNCGGCACCTTCATGAACGA (SEQ ID NO:541)
F180	nsa381R	GATCACCGAACCCGCGTCSNNGAACGGCACCTTCATGAA (SEQ ID NO:542)
D181	nsa382R	GCTGATCACCGAACCCGCSNNGAAGAACGGCACCTTCAT (SEQ ID NO:543)
A182	nsa383R	GGTGCTGATCACCGAACCSNNGTCGAAGAACGGCACCTT (SEQ ID NO:544)
G183	nsa384R	GTCGGTGCTGATCACCGASNNCGCGTCGAAGAACGGCAC (SEQ ID NO:545)
S184	nsa385R	GCCGTCGGTGCTGATCACSNNACCCGCGTCGAAGAACGG (SEQ ID NO:546)
V185	nsa386R	GACGCCGTCGGTGCTGATSNNCGAACCCGCGTCGAAGAA (SEQ ID NO:547)
I186	nsa387R	GTCGACGCCGTCGGTGCTSNNCACCGAACCCGCGTCGAA (SEQ ID NO:548)
S187	nsa388R	TCCGTCGACGCCGTCGGTSNNGATCACCGAACCCGCGTC (SEQ ID NO:549)
T188	nsa389R	GATTCCGTCGACGCCGTCSNNGCTGATCACCGAACCCGC (SEQ ID NO:550)

D189	nsa390R	GTGGATTCCGTCGACGCCSNNGGTGCTGATCACCGAACC (SEQ ID NO:551)
G190	nsa391R	GAAGTGGATTCCGTCGACSNNGTCGGTGCTGATCACCGA (SEQ ID NO:552)
V191	nsa392R	GGTGAAGTGGATTCCGTCSNNGCCGTCGGTGCTGATCAC (SEQ ID NO:553)
D192	nsa393R	CTCGGTGAAGTGGATTCCSNNGACGCCGTCGGTGCTGAT (SEQ ID NO:554)
G193	nsa394R	GGCCTCGGTGAAGTGGATSNNGTCGACGCCGTCGGTGCT (SEQ ID NO:555)
I194	nsa181R	GTTGGCCTCGGTGAAGTGSNNTCCGTCGACGCCGTCGGT (SEQ ID NO:556)
H195	nsa396R	ATTGTTGGCCTCGGTGAASNNGATTCCGTCGACGCCGTC (SEQ ID NO:557)
F196	nsa182R	GCGATTGTTGGCCTCGGTSNNGTGGATTCCGTCGACGCC (SEQ ID NO:558)
T197	nsa398R	ATCGCGATTGTTGGCCTCSNNGAAGTGGATTCCGTCGAC (SEQ ID NO:559)
E198	nsa399R	GAGATCGCGATTGTTGGCSNNGGTGAAGTGGATTCCGTC (SEQ ID NO:560)
A199	nsa400R	CCCGAGATCGCGATTGTTSNNCTCGGTGAAGTGGATTCC (SEQ ID NO:561)
N200	nsa401R	CACCCCGAGATCGCGATTSNNGGCCTCGGTGAAGTGGAT (SEQ ID NO:562)
N201	nsa402R	GGCCACCCCGAGATCGCGSNNGTTGGCCTCGGTGAAGTG (SEQ ID NO:563)
R202	nsa403R	GAGGGCCACCCCGAGATCSNNATTGTTGGCCTCGGTGAA (SEQ ID NO:564)
D203	nsa404R	CGCGAGGGCCACCCCGAGSNNGCGATTGTTGGCCTCGGT (SEQ ID NO:565)
L204	nsa405R	TTCCGCGAGGGCCACCCCSNNATCGCGATTGTTGGCCTC (SEQ ID NO:566)
G205	nsa406R	CTGTTCCGCGAGGGCCACSNNGAGATCGCGATTGTTGGC (SEQ ID NO:567)
V206	nsa407R	CACCTGTTCCGCGAGGGCSNNCCCGAGATCGCGATTGTT (SEQ ID NO:568)
A207	nsa408R	CTGCACCTGTTCCGCGAGSNNCACCCCGAGATCGCGATT (SEQ ID NO:569)
L208	nsa409R	GCTCTGCACCTGTTCCGCSNNGGCCACCCCGAGATCGCG (SEQ ID NO:570)

A209	nsa410R	CAGGCTCTGCACCTGTTCSNNGAGGGCCACCCCGAGATC (SEQ ID NO:571)
E210	nsa411R	CAGCAGGCTCTGCACCTGSNNCGCGAGGGCCACCCCGAG (SEQ ID NO:572)
Q211	nsa412R	TTACAGCAGGCTCTGCACSNNTTCCGCGAGGGCCACCCC (SEQ ID NO:573)
V212	nsa413R	CTTTTACAGCAGGCTCTGSNNCTGTTCCGCGAGGGCCAC (SEQ ID NO:574)
Q213	nsa414R	GCCCTTTTACAGCAGGCTSNNCACCTGTTCCGCGAGGGC (SEQ ID NO:575)
S214	nsa415R	TTCGCCCTTTTACAGCAGSNNCTGCACCTGTTCCGCGAG (SEQ ID NO:576)
L215	nsa416R	GAATTCGCCCTTTTACAGSNNGCTCTGCACCTGTTCCGC (SEQ ID NO:577)
L216	nsa417R	GCAGAATTCGCCCTTTTASNNCAGGCTCTGCACCTGTTC (SEQ ID NO:578)

QC Method to Create Site-Saturation Libraries

The QC reaction consisted of 40.25 µL of sterile distilled H_2O, 5 µL of PfuTurbo 10x buffer from the kit, 1µL dNTPs from the kit, 1.25 µL of forward primer (100ng/µL), 1.25 µL reverse primer (100ng/µL), 0.25 µL of pMSAT-NcoI miniprep DNA as template (~50ng), and 1 µL of PfuTurbo from the kit, for a total of 50 µL. The cycling conditions were 95°C for 1min, once, followed by 19-20 cycles of 95°C for 30 to 45 sec, 55°C for 1min, and 68°C for 5 to 8 min. To analyze the reaction, 5µL of the reaction was run on a 0.8% E-gel (Invitrogen) upon completion. Next, *Dpn*I digestion was carried out twice sequentially, with 1 µL and 0.5 µL of enzyme at 37°C for 2 to 8 hours. A negative control was carried out under similar conditions, but without any primers. Then, 1 µL of the *Dpn*I-digested reaction product was transformed into 50 µL of one-shot TOP10 electrocompetent cells (Invitrogen) using a BioRad electroporator. Then, 300 µL of SOC provided with the TOP10 cells (Invitrogen) were added to the electroporated cells and incubated with shaking for 1 hour before plating on LA plates containing 10ppm kanamycin. The plates were incubated at 37°C overnight. After this incubation, 96 colonies from each of the libraries (*i.e.*, each site) were inoculated in 200µL of LB containing 10-50ppm of kanamycin in 96-well microtiter plates. The plates were frozen at –80°C after addition of glycerol to 20% final concentration, and they were used for high throughput sequencing at Genaissance with the M13F and M13R primers.

QCMS Method to Create Site-Saturation Libraries

The QCMS reaction consisted of 19.25 µL of sterile distilled H_2O, 2.5 µL of 10x buffer from the kit, 1µL dNTPs from the kit, 1µL of 5' phosphorylated forward primer (100ng/µL), 0.25 µL of pMSAT-NcoI miniprep DNA as template (~50ng), and 1µL of the enzyme blend from the kit for a total of 25 µL. The cycling conditions were 95°C for 1min once, followed by 30 cycles of 95°C for 1min, 55°C for 1min, and 68°C for 8 min. To analyze the reaction product, 5µL of the reaction were run on a 0.8% E-gel (Invitrogen) upon completion. Next, *Dpn*I digestion was carried out twice sequentially, with 0.5 µL of enzyme at 37°C for 2 to 8 hours. The controls, transformation, and sequencing was performed as for the QC method described above.

Details of Screening Plate Preparation

Using a sterilized stamping tool with 96 pins, the frozen clones from each sequenced library plate were stamped on to a large LA plate containing 10ppm kanamycin. The plate was then incubated overnight at 37°C. Individual mutant clones each representing each one of the 19 substitutions (or as many that were obtained) were inoculated into a Costar 96-well plate containing 195µL of LB made with 2 fold greater yeast extract and 10ppm kanamycin. Each mutant clone for a given site was inoculated in quadruplicate. The plate was grown at 37°C and 225 rpm shaking for 18 hrs in a humidified chamber. In a separate 96-well plate, 26µL of BugBuster (Novagen) with DNase were added to each well. Next, 125µL of the library clone cultures were added to the BugBuster-containing plate in corresponding wells and the plate was frozen at –80°C.

The plate was thawed, frozen and thawed again before use of the lysates in the peracid formation and peracid hydrolysis assays described herein.

Combinatorial Libraries and Mutants

From the screening of the single site-saturation libraries, the important sites and substitutions were identified and combined in different combinatorial libraries. For example, libraries described in Table 8-3 were created using the following sites and substitutions:

L12C, Q, G
T25S, G, P
L53H, Q, G, S
S54V, L, A, P, T, R
A55G, T
R67T, Q, N, G, E, L, F
K97R
V125S, G, R, A, P
F154Y
F196G

TABLE 8-3. Libraries

Library	Description	Parent Template	Method
NSAA1	L12G S54(NNS)	L12G	QC
NSAA2	S54V L12(NNS)	S54V	QC
NSAA3	L12(NNS) S54(NNS)	WT	QCMS
NSAB1	S54V T25(NNS)	S54V	QC
NSAB2	S54V R67(NNS)	S54V	QC
NSAB3	S54V V125(NNS)	S54V	QC
NSAB4	L12I S54V T25(NNS)	L12I S54V	QC
NSAB5	L12I S54V R67(NNS)	L12I S54V	QC
NSAB6	L12I S54V V125(NNS)	L12I S54V	QC
NSAC1	S54(NNS) R67(NNS) V125(NNS)	WT	QCMS
NSAC2	43 primer library; 10 sites (100ng total primers)	S54V	QCMS
NSAC3	same as nsaC2 but 300ng total primers	S54V	QCMS
NSAC4	32 primer library, 8 sites (100ng total primers)	S54V	QCMS
NSAC5	same as nsaC4 but 300ng total primers	S54V	QCMS
NSAC6	8 primers, 7 substitutions, 5 sites (100ng total primers)	S54V	QCMS
NSAC7	same as nsaC6 but 300ng total primers	S54V	QCMS

*NNS indicates site-saturation library
**All parent templates were derived from the pMSAT-NcoI plasmid and contained mutations at the indicated codons with in the *M. smegmatis* perhydrolase gene

The QC or QCMS methods were used to create the combinations. The QC reaction was carried out as described above, with the exception being the template plasmid, which consisted of 0.25μL of miniprep DNA of the L12G mutant, S54V mutant, or the L12I S54V double mutant plasmid derived from pMSAT-NcoI. The QCMS reaction was also carried out as described above, with the exception of template and primers. In this case, 0.25µL of the pMSAT-NcoI template were used for NSAC1 and NSAA3 or S54V template for NSAC2-C7 libraries. The NSAA3 and the NSAC1 libraries were made using 100 ng of each of the primers shown in the Table 8-4. The NSAC2, NSAC4, and NSAC6 libraries were made with a total of 100ng of all primers (all primers being equimolar), and NSAC3, NSAC5, NSAC7 libraries were made with a total of 300ng of all primers (all primers being approximately equimolar)

Table 8-4. Libraries

Libraries	Primer Name	Primer Sequence
NSAC1	S54NNS-FP	gtgatcgaggagggactgnnsgcgcgcaccaccaacatc (SEQ ID NO:579)
NSAC1	R67NNS-FP	acgaccccaccgatccgnnsctcaacggcgcgagctac (SEQ ID NO:580)
NSAC1	V125NNS-FP	ctcaccagcgcgggcggcnnsggcaccacgtacccggca (SEQ ID NO:581)
NSAC2-C5	L12C	ctgtgtttcggtgattccTGCacctggggctgggtcccc (SEQ ID NO:582)
NSAC2-C7	L12Q	ctgtgtttcggtgattccCAGacctggggctgggtcccc (SEQ ID NO:583)
NSAC2-C5	L12I	ctgtgtttcggtgattccATCacctggggctgggtcccc (SEQ ID NO:584)
NSAC2-C3	L12M	ctgtgtttcggtgattccATGacctggggctgggtcccc (SEQ ID NO:585)
NSAC2-C3	L12T	ctgtgtttcggtgattccACGacctggggctgggtcccc (SEQ ID NO:586)
NSAC2-C5	T25S	gtcgaagacggggcacccAGCgagcggttcgcccccgac (SEQ ID NO:587)
NSAC2-C5	T25G	gtcgaagacggggcacccGGCgagcggttcgcccccgac (SEQ ID NO:588)
NSAC2-C3	T25P	gtcgaagacggggcacccCCGgagcggttcgcccccgac (SEQ ID NO:589)
NSAC2-C7	L53H	gaggtgatcgaggagggaCACagcgcgcgcaccaccaac (SEQ ID NO:590)
NSAC2-C3	L53Q	gaggtgatcgaggagggaCAGagcgcgcgcaccaccaac (SEQ ID NO:591)
NSAC2-C3	L53G	gaggtgatcgaggagggaGGCagcgcgcgcaccaccaac (SEQ ID NO:592)
NSAC2-C3	L53S	gaggtgatcgaggagggaAGCagcgcgcgcaccaccaac (SEQ ID NO:593)
NSAC2-C7	L53HS54V	gaggtgatcgaggagggaCACGTGgcgcgcaccaccaac (SEQ ID NO:594)
NSAC2-C3	L53QS54V	gaggtgatcgaggagggaCAGGTGgcgcgcaccaccaac (SEQ ID NO:595)
NSAC2-C3	L53GS54V	gaggtgatcgaggagggaGGCGTGgcgcgcaccaccaac (SEQ ID NO:596)
NSAC2-C3	L53SS54V	gaggtgatcgaggagggaAGCGTGgcgcgcaccaccaac (SEQ ID NO:597)
NSAC2-C7	S54V	gtgatcgaggagggactgGTGgcgcgcaccaccaacatc (SEQ ID NO:598)
NSAC2-C5	S54L	gtgatcgaggagggactgCTGgcgcgcaccaccaacatc (SEQ ID NO:599)
NSAC2-C5	A55G	atcgaggagggactgagcGGCcgcaccaccaacatcgac (SEQ ID NO:600)

NSAC2-C5	A55T	atcgaggagggactgagcACGcgcaccaccaacatcgac (SEQ ID NO:601)
NSAC2-C5	A55GS54V	atcgaggagggactgGTGGGCcgcaccaccaacatcgac (SEQ ID NO:602)
NSAC2-C5	A55TS54V	atcgaggagggactgGTGACGcgcaccaccaacatcgac (SEQ ID NO:603)
NSAC2-C5	R67T	gacgaccccaccgatccgACGctcaacggcgcgagctac (SEQ ID NO:604)
NSAC2-C5	R67Q	gacgaccccaccgatccgCAGctcaacggcgcgagctac (SEQ ID NO:605)
NSAC2-C7	R67N	gacgaccccaccgatccgAACctcaacggcgcgagctac (SEQ ID NO:606)
NSAC2-C5	K97R	ctgggcaccaacgacaccCGCgcctacttccggcgcacc (SEQ ID NO:607)
NSAC2-C5	V125S	ctcaccagcgcgggcggcAGCggcaccacgtacccggca (SEQ ID NO:608)
NSAC2-C7	V125G	ctcaccagcgcgggcggcGGCggcaccacgtacccggca (SEQ ID NO:609)
NSAC2-C5	V125R	ctcaccagcgcgggcggcCGCggcaccacgtacccggca (SEQ ID NO:610)
NSAC2-C5	V125A	ctcaccagcgcgggcggcGCGggcaccacgtacccggca (SEQ ID NO:611)
NSAC2-C5	V125P	ctcaccagcgcgggcggcCCGggcaccacgtacccggca (SEQ ID NO:612)
NSAC2-C3	F154Y	ccctggttccagttgatcTACgagggcggcgagcagaag (SEQ ID NO:613)
NSAC2-C3	F196G	ggcgtcgacggaatccacGGCaccgaggccaacaatcgc (SEQ ID NO:614)
NSAC2-C7	R67G-re	gacgaccccaccgatccgGGCctcaacggcgcgagctac (SEQ ID NO:615)
NSAC2-C5	R67E-re	gacgaccccaccgatccgGAGctcaacggcgcgagctac (SEQ ID NO:616)
NSAC2-C5	R67F-re	gacgaccccaccgatccgTTCctcaacggcgcgagctac (SEQ ID NO:617)
NSAC2-C5	R67L-re	gacgaccccaccgatccgCTGctcaacggcgcgagctac (SEQ ID NO:618)
NSAC2-C5	S54P	gtgatcgaggagggactgCCGgcgcgcaccaccaacatc (SEQ ID NO:619)
NSAC2-C5	S54R	gtgatcgaggagggactgCGCgcgcgcaccaccaacatc (SEQ ID NO:620)
NSAC2-C5	S54G	gtgatcgaggagggactgGGCgcgcgcaccaccaacatc (SEQ ID NO:621)
NSAC2-C5	S54T	gtgatcgaggagggactgACGgcgcgcaccaccaacatc (SEQ ID NO:622)
NSAC2-C7	S54I	gtgatcgaggagggactgATCgcgcgcaccaccaacatc (SEQ ID NO:623)
NSAC2-C5	S54K	gtgatcgaggagggactgAAGgcgcgcaccaccaacatc (SEQ ID NO:624)

Screening of Combinatorial Libraries and Mutants

For each of the NSAB1-B6 libraries, a 96-well plate full of clones was first sequenced. Once the sequencing results were analyzed, the mutants obtained for each library were inoculated in quadruplicate, similar to the site-saturation libraries described above. For the NSAC1-C7 libraries, 96 colonies per/plate/library were initially inoculated, and each plate was screened without sequencing. Upon screening, some libraries looked better than others. Several plates for each of the NSAC1, C2, C4, C6 libraries were screened. The "winners" from these single isolate screening plates were then streaked out for singles or directly screened in quadruplicate just like the site-saturation libraries (*i.e.*, as described above). Only the "winners" identified were sequenced.

EXAMPLE 9

Improved Properties of Multiply Mutated Perhydrolase Variants

In this Example, experiments conducted to assess the properties of multiply-mutated perhydrolase variants are described. In these experiments, combinatorial mutants obtained from combinatorial libraries were tested in their performance in perhydrolysis, peracid hydrolysis and perhydrolysis to hydrolysis ratio. These parameters were measured in the HPLC or ABTS assays described in Example 2, above. Combinatorial variants tested were:

L12I S54V,
L12M S54T,
L12T S54V,
L12Q T25S S54V,
L53H S54V,
S54P V125R,
S54V V125G,
S54V F196G,
S54V K97R V125G, and
A55G R67T K97R V125G,

As is indicated in Table 9-1 below, all of these variants were better than wild type enzyme in at least one of the properties of interest.

Table 9-1 Results for Multiple Variants	
Multiple Variant	**Fold-Improvement in Property**

	Perhydrolysis	Peracid Hydrolysis	Ratio
L12I S54V	2	2.5	
L12M S54T	1.6	3	
L12T S54V	1.5	2.5	
L12Q T25S S54V		4 to 5	
L53H S54V	2		4 to 5
S54P V125R			4
S54V V125G	2		4
S54V F196G			2
S54V K97R V125G	2		
A55G R67T K97R V125G	1.6		4 to 5

EXAMPLE 10
PAF and PAD Assays of Perhydrolase Variants

In this Example, assay results for PAF and PAD testing of perhydrolase variants are provided. The tests were conducted as described in Example 1, above. In addition, Tables are provided in which the protein expression of the variant was greater than wild-type under the same culture conditions (described herein). These results are indicated as the "protein performance index." Thus, a number greater than "1" in the protein performance index indicates that more protein was made for the particular variant than the wild-type. In the following Tables, "WT" indicates the wild-type amino acid residue; "Pos" indicates the position in the amino acid sequence; "Mut." and "Var" indicate the amino acid residue substituted at that particular position; "prot." indicates "protein; and "Perf. Ind" indicates the performance index.

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
3	K003Y	Y	1.058244
3	K003I	I	1.053242
3	K003L	L	1.038686
3	K003T	T	1.009071
3	K003H	H	1.00528
4	R004Q	Q	1.025332
5	I005T	T	1.12089
5	I005S	S	1.023576
6	L006V	V	1.072388
6	L006I	I	1.066182
6	L006T	T	1.062078
7	C007K	K	2.687956
7	C007Y	Y	2.08507
7	C007I	I	1.758096
7	C007H	H	1.731475
7	C007A	A	1.423943
7	C007G	G	1.393781
7	C007M	M	1.126028
10	D010L	L	3.97014
10	D010W	W	3.179778
10	D010K	K	2.133852
10	D010Y	Y	1.508981
10	D010T	T	1.473387
10	D010I	I	1.281927
12	L012Q	Q	2.651732
12	L012C	C	2.289224
12	L012A	A	1.100171
15	G015A	A	1.543799
15	G015S	S	1.05273
17	V017G	G	1.173641
17	V017R	R	1.09735
17	V017A	A	1.012116
18	P018Y	Y	1.332844
18	P018N	N	1.331062
18	P018C	C	1.261104
18	P018E	E	1.217708
18	P018V	V	1.185736
18	P018R	R	1.16328
18	P018Q	Q	1.124133
18	P018H	H	1.120443
18	P018G	G	1.068272
19	V019G	G	1.317001
19	V019S	S	1.235759
19	V019R	R	1.025471
19	V019L	L	1.002833
21	D021K	K	1.062138
21	D021W	W	1.040173
22	G022A	A	1.554264
22	G022T	T	1.032118
22	G022S	S	1.022133
25	T025G	G	1.857878
25	T025S	S	1.59954
25	T025A	A	1.327579
25	T025I	I	1.019417
26	E026M	M	2.002044
26	E026A	A	1.927099
26	E026R	R	1.484814
26	E026K	K	1.464368
26	E026T	T	1.441939
26	E026C	C	1.403045

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
26	E026V	V	1.392881
26	E026N	N	1.366419
26	E026H	H	1.329562
26	E026L	L	1.295378
26	E026G	G	1.283477
26	E026S	S	1.271403
26	E026W	W	1.251752
27	R027K	K	1.215697
28	F028M	M	1.331874
28	F028A	A	1.269493
28	F028W	W	1.156698
28	F028L	L	1.08849
28	F028S	S	1.046063
29	A029W	W	1.912244
29	A029V	V	1.799733
29	A029R	R	1.757225
29	A029Y	Y	1.697554
29	A029G	G	1.595061
29	A029S	S	1.486877
29	A029T	T	1.424584
29	A029E	E	1.115768
29	A029C	C	1.07522
30	P030K	K	1.207673
30	P030R	R	1.164892
30	P030V	V	1.063047
30	P030T	T	1.05383
30	P030A	A	1.045476
30	P030S	S	1.031747
30	P030Q	Q	1.013468
30	P030H	H	1.012332
30	P030E	E	1.006761
31	D031W	W	1.834044
31	D031L	L	1.810564
31	D031T	T	1.450556
31	D031G	G	1.441703
31	D031F	F	1.438268
31	D031N	N	1.339422
31	D031V	V	1.280091
31	D031A	A	1.240923
31	D031R	R	1.222181
31	D031S	S	1.152736
31	D031E	E	1.132795
31	D031Q	Q	1.069797
32	V032K	K	1.08606
32	V032R	R	1.045435
33	R033S	S	1.000491
36	G036I	I	1.320156
36	G036K	K	1.265563
36	G036L	L	1.237473
38	L038L	L	6.528092
38	L038V	V	5.735873
38	L038C	C	4.182031
38	L038K	K	4.135067
38	L038A	A	3.844719
38	L038S	S	2.467764
40	Q040K	K	2.613726
40	Q040I	I	2.576806
40	Q040W	W	2.394926
40	Q040L	L	2.144687
40	Q040T	T	2.006487

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
40	Q040R	R	1.885154
40	Q040Y	Y	1.825366
40	Q040G	G	1.785768
40	Q040S	S	1.565973
40	Q040N	N	1.528677
40	Q040D	D	1.16151
40	Q040E	E	1.075259
41	Q041K	K	1.381385
41	Q041R	R	1.190317
41	Q041W	W	1.141041
41	Q041H	H	1.123719
41	Q041S	S	1.107641
41	Q041Y	Y	1.091652
41	Q041V	V	1.070265
41	Q041A	A	1.032945
41	Q041L	L	1.000416
42	L042K	K	2.463086
42	L042W	W	2.056507
42	L042H	H	1.917245
42	L042R	R	1.378137
42	L042G	G	1.172748
42	L042T	T	1.079826
42	L042F	F	1.072948
43	G043A	A	1.49082
43	G043C	C	1.47701
43	G043K	K	1.424919
43	G043M	M	1.371202
43	G043Y	Y	1.262703
43	G043E	E	1.250311
43	G043L	L	1.216516
43	G043R	R	1.215829
43	G043S	S	1.178103
43	G043H	H	1.169457
43	G043P	P	1.080176
44	A044F	F	2.84399
44	A044V	V	2.133682
44	A044C	C	1.796096
44	A044L	L	1.607918
44	A044W	W	1.395243
44	A044M	M	1.199028
45	D045K	K	1.342858
45	D045T	T	1.268367
45	D045R	R	1.158768
45	D045W	W	1.145157
45	D045S	S	1.133098
45	D045G	G	1.12761
45	D045H	H	1.127539
45	D045F	F	1.11152
45	D045L	L	1.054441
45	D045V	V	1.050576
45	D045Q	Q	1.04498
45	D045A	A	1.037993
46	F046E	E	1.247552
46	F046D	D	1.174794
46	F046G	G	1.016913
46	F046K	K	1.003326
47	E047R	R	2.448525
47	E047T	T	1.960505
47	E047P	P	1.361173
47	E047S	S	1.278809

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
47	E047H	H	1.266229
47	E047G	G	1.197541
47	E047K	K	1.19183
47	E047F	F	1.092281
47	E047I	I	1.030029
49	I049G	G	1.342918
49	I049H	H	1.265204
49	I049S	S	1.238211
49	I049K	K	1.230871
49	I049V	V	1.203314
49	I049L	L	1.136805
49	I049Y	Y	1.068104
49	I049R	R	1.052285
49	I049E	E	1.015762
49	I049M	M	1.00526
50	E050L	L	1.191901
50	E050M	M	1.178039
50	E050A	A	1.124087
51	E051V	V	1.471315
51	E051A	A	1.279983
51	E051G	G	1.217963
51	E051T	T	1.182792
51	E051L	L	1.112889
51	E051I	I	1.072835
53	L053H	H	5.05321
53	L053Q	Q	1.480206
53	L053G	G	1.317357
53	L053S	S	1.161011
53	L053T	T	1.019146
54	S054P	P	5.198689
54	S054I	I	4.775938
54	S054V	V	4.722033
54	S054A	A	3.455902
54	S054R	R	3.375793
54	S054L	L	2.015828
54	S054T	T	1.459971
54	S054K	K	1.438715
54	S054G	G	1.429605
54	S054C	C	1.259773
54	S054Q	Q	1.03365
55	A055G	G	1.694814
55	A055T	T	1.692885
57	T057S	S	1.633613
57	T057R	R	1.605072
57	T057V	V	1.281788
57	T057I	I	1.189062
59	N059W	W	1.035044
59	N059R	R	1.002315
60	I060H	H	1.02415
60	I060R	R	1.003947
61	D061H	H	1.439407
61	D061S	S	1.259714
61	D061R	R	1.105425
61	D061I	I	1.076937
61	D061F	F	1.00566
62	D062E	E	1.019293
63	P063G	G	1.709657
63	P063T	T	1.499483
63	P063M	M	1.460336
63	P063S	S	1.416192

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
63	P063K	K	1.404615
63	P063A	A	1.347541
63	P063Y	Y	1.346046
63	P063W	W	1.34587
63	P063V	V	1.313631
63	P063R	R	1.310696
63	P063F	F	1.246299
63	P063L	L	1.146416
63	P063Q	Q	1.093179
64	T064G	G	1.234467
64	T064S	S	1.114348
65	D065A	A	1.312312
65	D065S	S	1.166849
65	D065H	H	1.096335
66	P066R	R	1.846257
66	P066V	V	1.828926
66	P066H	H	1.589631
66	P066I	I	1.588219
66	P066G	G	1.499901
66	P066Q	Q	1.463705
66	P066T	T	1.410091
66	P066S	S	1.390845
66	P066Y	Y	1.330685
66	P066L	L	1.137635
66	P066N	N	1.122261
67	R067N	N	1.580401
67	R067G	G	1.390129
67	R067T	T	1.284643
67	R067F	F	1.25763
67	R067L	L	1.203316
67	R067Q	Q	1.164899
67	R067W	W	1.066028
67	R067E	E	1.044676
67	R067P	P	1.012761
68	L068E	E	1.435218
68	L068W	W	1.209193
68	L068I	I	1.125898
68	L068G	G	1.092454
68	L068V	V	1.088042
68	L068H	H	1.051612
68	L068T	T	1.032331
69	N069V	V	1.989028
69	N069K	K	1.71908
69	N069R	R	1.493163
69	N069I	I	1.469946
69	N069H	H	1.357968
69	N069T	T	1.351305
69	N069L	L	1.299547
69	N069S	S	1.205171
69	N069G	G	1.19653
69	N069Q	Q	1.074622
69	N069W	W	1.049602
69	N069C	C	1.048373
71	A071S	S	1.751794
71	A071T	T	1.700442
71	A071H	H	1.697558
71	A071G	G	1.58881
71	A071I	I	1.507841
71	A071E	E	1.445699
71	A071K	K	1.441146

Table 10-1. PAF Assay Results

Position	WT/Pos/Mutation	Variant	PAF Perf. Ind.
71	A071R	R	1.401499
71	A071N	N	1.232241
71	A071L	L	1.231991
71	A071F	F	1.127538
71	A071C	C	1.00977
72	S072L	L	1.257945
72	S072H	H	1.208899
72	S072G	G	1.198197
72	S072T	T	1.10065
72	S072V	V	1.080089
72	S072Y	Y	1.066178
73	Y073R	R	1.2555
73	Y073Q	Q	1.23429
73	Y073S	S	1.165683
73	Y073K	K	1.070678
76	S076P	P	1.229172
77	C077T	T	1.120603
77	C077V	V	1.052586
77	C077G	G	1.013806
78	L078G	G	4.975852
78	L078H	H	4.824004
78	L078E	E	3.007159
78	L078N	N	2.683604
78	L078T	T	1.867711
78	L078Q	Q	1.726942
78	L078V	V	1.534239
78	L078I	I	1.434206
78	L078Y	Y	1.387889
79	A079H	H	1.927914
79	A079L	L	1.796126
79	A079I	I	1.592463
79	A079M	M	1.499635
79	A079N	N	1.475806
79	A079Q	Q	1.472484
79	A079R	R	1.465943
79	A079W	W	1.270538
79	A079T	T	1.169146
79	A079E	E	1.123457
80	T080C	C	1.310752
80	T080V	V	1.230659
80	T080G	G	1.160318
80	T080A	A	1.000722
82	L082P	P	1.456374
82	L082G	G	1.379439
82	L082R	R	1.339485
82	L082H	H	1.332844
82	L082K	K	1.1909
82	L082T	T	1.17992
82	L082I	I	1.171013
82	L082S	S	1.153417
82	L082V	V	1.019854
83	P083K	K	1.369406
83	P083G	G	1.313431
83	P083H	H	1.265876
83	P083R	R	1.194464
83	P083S	S	1.171208
84	L084K	K	1.099089
84	L084H	H	1.008187
85	D085Q	Q	3.093245
85	D085R	R	2.379647

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
85	D085S	S	2.284009
85	D085H	H	1.548556
85	D085N	N	1.539497
85	D085G	G	1.413812
85	D085T	T	1.329395
85	D085E	E	1.117228
85	D085F	F	1.008028
86	L086A	A	1.376284
86	L086C	C	1.156625
86	L086G	G	1.145834
95	D095E	E	2.044825
96	T096S	S	1.044425
97	K097R	R	2.798748
97	K097Q	Q	1.136975
100	F100W	W	1.082799
100	F100E	E	1.0116
101	R101K	K	1.244945
103	T103W	W	1.261503
103	T103Y	Y	1.193299
103	T103G	G	1.113343
103	T103K	K	1.093573
103	T103I	I	1.076338
103	T103L	L	1.050734
104	P104H	H	2.837034
104	P104T	T	2.696977
104	P104G	G	2.672719
104	P104V	V	2.585315
104	P104S	S	2.481687
104	P104I	I	2.431309
104	P104W	W	2.051785

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
104	P104C	C	1.951282
104	P104E	E	1.837373
104	P104F	F	1.785718
104	P104N	N	1.624722
104	P104R	R	1.618032
104	P104Q	Q	1.343174
104	P104M	M	1.093185
105	L105P	P	1.713219
105	L105C	C	1.557999
105	L105F	F	1.295759
105	L105W	W	1.283998
105	L105G	G	1.078743
106	D106K	K	1.278457
106	D106L	L	1.198148
106	D106G	G	1.178297
106	D106H	H	1.090134
106	D106E	E	1.084931
106	D106T	T	1.061622
106	D106I	I	1.036191
106	D106F	F	1.021513
106	D106C	C	1.005553
107	I107E	E	2.551108
107	I107S	S	2.044692
107	I107N	N	1.810584
107	I107G	G	1.764761
107	I107V	V	1.001703
108	A108L	L	1.407382
108	A108T	T	1.050964
109	L109N	N	1.523277
109	L109W	W	1.296964

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
109	L109Q	Q	1.182653
109	L109Y	Y	1.155328
109	L109I	I	1.053129
109	L109D	D	1.003394
111	M111K	K	1.977248
111	M111I	I	1.949343
111	M111L	L	1.546317
111	M111T	T	1.489808
111	M111F	F	1.467344
111	M111V	V	1.466478
111	M111Y	Y	1.42589
111	M111S	S	1.031939
112	S112L	L	1.027928
112	S112H	H	1.001485
113	V113L	L	1.503622
113	V113H	H	1.339003
113	V113K	K	1.192607
113	V113R	R	1.133751
113	V113Y	Y	1.113256
113	V113F	F	1.045057
113	V113Q	Q	1.032496
115	V115W	W	1.234
115	V115T	T	1.145757
115	V115L	L	1.117398
115	V115G	G	1.089596
115	V115I	I	1.050387
115	V115Y	Y	1.032052
116	T116G	G	1.095496
116	T116A	A	1.006702
117	Q117H	H	2.327857
117	Q117T	T	2.233854
117	Q117Y	Y	2.227983
117	Q117W	W	2.155359
117	Q117V	V	2.154646
117	Q117G	G	2.080223
117	Q117A	A	2.048752
117	Q117S	S	1.949232
117	Q117F	F	1.573776
117	Q117R	R	1.564466
117	Q117M	M	1.541944
117	Q117E	E	1.145341
118	V118Y	Y	1.25067
118	V118K	K	1.125917
118	V118G	G	1.083422
120	T120S	S	1.089798
121	S121L	L	1.348931
121	S121W	W	1.333741
121	S121R	R	1.25879
121	S121K	K	1.241105
121	S121G	G	1.204547
121	S121C	C	1.177769
121	S121N	N	1.143954
121	S121T	T	1.132507
121	S121A	A	1.120633
121	S121V	V	1.120454
122	A122H	H	1.137861
122	A122I	I	1.133601
122	A122T	T	1.083131
122	A122K	K	1.082552
122	A122V	V	1.041449

Table 10-1. PAF Assay Results

Position	WT/Pos/ Mutation	Variant	PAF Perf. Ind.
122	A122S	S	1.031411
124	G124L	L	1.91642
124	G124I	I	1.853337
124	G124T	T	1.63716
124	G124H	H	1.588068
124	G124V	V	1.441979
124	G124F	F	1.320782
124	G124S	S	1.269245
124	G124Y	Y	1.234423
124	G124R	R	1.144212
124	G124Q	Q	1.123498
125	V125G	G	2.948291
125	V125S	S	1.942881
125	V125A	A	1.689696
125	V125P	P	1.50166
125	V125R	R	1.301534
125	V125D	D	1.238852
125	V125Y	Y	1.080394
125	V125I	I	1.010779
126	G126T	T	1.577938
126	G126P	P	1.171092
126	G126L	L	1.169527
127	T127H	H	1.57251
127	T127V	V	1.073821
127	T127I	I	1.063668
127	T127S	S	1.046984
128	T128L	L	1.064623
128	T128K	K	1.062947
148	P148V	V	2.426937
148	P148K	K	1.786508
148	P148L	L	1.638438
148	P148A	A	1.637334
148	P148R	R	1.509086
148	P148T	T	1.501359
148	P148Y	Y	1.459512
148	P148S	S	1.45564
148	P148E	E	1.417449
148	P148F	F	1.367568
148	P148Q	Q	1.334517
148	P148D	D	1.030185
150	F150L	L	1.290835
150	F150E	E	1.228159
153	I153K	K	1.618543
153	I153H	H	1.464262
153	I153T	T	1.271928
153	I153L	L	1.270149
153	I153F	F	1.227821
153	I153A	A	1.194659
154	F154Y	Y	1.323693
196	F196H	H	1.774774
196	F196L	L	1.768072
196	F196C	C	1.738263
196	F196M	M	1.647608
196	F196G	G	1.590716
196	F196S	S	1.577837
196	F196Y	Y	1.414589
196	F196V	V	1.395387
196	F196I	I	1.320955
196	F196W	W	1.014435

The following Table provides variants with PAF results that were better than those observed for wild-type M. smegmatis perhydrolase. In this Table, the middle column indicates the amino acid residue in the wild-type perhydrolase (WT), followed by the position number and the variant amino acid in that position (Var).

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
2	A002W	1.75
2	A002D	1.30
2	A002F	1.24
2	A002I	1.18
2	A002G	1.15
2	A002S	1.01
3	K003Y	1.06
3	K003I	1.05
3	K003L	1.04
3	K003T	1.01
3	K003H	1.01
4	R004Q	1.03
5	I005T	1.12
5	I005S	1.02
6	L006V	1.07
6	L006I	1.07
6	L006T	1.06
7	C007K	2.69
7	C007Y	2.09
7	C007I	1.76
7	C007H	1.73
7	C007A	1.42
7	C007G	1.39
7	C007M	1.13
8	F008R	1.43
8	F008V	1.18
8	F008G	1.09
8	F008H	1.02
10	D010L	3.97
10	D010W	3.18
10	D010K	2.13
10	D010Y	1.51
10	D010T	1.47
10	D010I	1.28
12	L012Q	2.65
12	L012C	2.29
12	L012A	1.10
15	G015A	1.54
15	G015S	1.05
17	V017G	1.17
17	V017R	1.10
17	V017A	1.01
18	P018Y	1.33
18	P018N	1.33
18	P018C	1.26
18	P018E	1.22
18	P018V	1.19
18	P018R	1.16
18	P018Q	1.12
18	P018H	1.12
18	P018G	1.07
19	V019G	1.32

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
19	V019S	1.24
19	V019R	1.03
19	V019L	1.00
20	E020W	2.94
20	E020G	2.36
20	E020T	2.22
20	E020L	2.20
20	E020H	2.17
20	E020V	2.11
20	E020S	2.01
20	E020C	1.57
20	E020N	1.40
20	E020A	1.29
20	E020Q	1.27
21	D021K	1.58
21	D021W	1.55
21	D021L	1.46
21	D021A	1.46
21	D021G	1.37
21	D021Y	1.30
21	D021F	1.30
21	D021S	1.24
22	G022A	1.55
22	G022T	1.03
22	G022S	1.02
25	T025G	1.86
25	T025S	1.60
25	T025A	1.33
25	T025I	1.02
26	E026M	2.00
26	E026A	1.93
26	E026R	1.48
26	E026K	1.46
26	E026T	1.44
26	E026C	1.40
26	E026V	1.39
26	E026N	1.37
26	E026H	1.33
26	E026L	1.30
26	E026G	1.28
26	E026S	1.27
26	E026W	1.25
27	R027K	1.22
28	F028M	1.33
28	F028A	1.27
28	F028W	1.16
28	F028L	1.09
28	F028S	1.05
29	A029W	1.91
29	A029V	1.80
29	A029R	1.76
29	A029Y	1.70
29	A029G	1.60
29	A029S	1.49
29	A029T	1.42
29	A029E	1.12
29	A029C	1.08
30	P030K	1.21
30	P030R	1.16
30	P030V	1.06
30	P030T	1.05
30	P030A	1.05
30	P030S	1.03
30	P030Q	1.01

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
30	P030H	1.01
30	P030E	1.01
31	D031W	1.83
31	D031L	1.81
31	D031T	1.45
31	D031G	1.44
31	D031F	1.44
31	D031N	1.34
31	D031V	1.28
31	D031A	1.24
31	D031R	1.22
31	D031S	1.15
31	D031E	1.13
31	D031Q	1.07
32	V032K	1.09
32	V032R	1.05
33	R033S	1.00
36	G036I	1.32
36	G036K	1.27
36	G036L	1.24
37	V037S	1.40
37	V037I	1.26
37	V037A	1.25
37	V037H	1.21
37	V037L	1.16
37	V037C	1.09
37	V037T	1.05
39	A039L	1.43
39	A039K	1.36
39	A039Y	1.36
39	A039I	1.26
39	A039T	1.26
39	A039W	1.23
39	A039V	1.21
39	A039G	1.17
39	A039R	1.17
39	A039E	1.09
40	Q040K	2.61
40	Q040I	2.58
40	Q040W	2.39
40	Q040L	2.14
40	Q040T	2.01
40	Q040R	1.89
40	Q040Y	1.83
40	Q040G	1.79
40	Q040S	1.57
40	Q040N	1.53
40	Q040D	1.16
40	Q040E	1.08
41	Q041K	1.38
41	Q041R	1.19
41	Q041W	1.14
41	Q041H	1.12
41	Q041S	1.11
41	Q041Y	1.09
41	Q041V	1.07
41	Q041A	1.03
41	Q041L	1.00
42	L042K	2.46
42	L042W	2.06
42	L042H	1.92
42	L042R	1.38
42	L042G	1.17
42	L042T	1.08

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
42	L042F	1.07
43	G043A	1.49
43	G043C	1.48
43	G043K	1.42
43	G043M	1.37
43	G043Y	1.26
43	G043E	1.25
43	G043L	1.22
43	G043R	1.22
43	G043S	1.18
43	G043H	1.17
43	G043P	1.08
44	A044F	2.84
44	A044V	2.13
44	A044C	1.80
44	A044L	1.61
44	A044W	1.40
44	A044M	1.20
45	D045K	1.34
45	D045T	1.27
45	D045R	1.16
45	D045W	1.15
45	D045S	1.13
45	D045G	1.13
45	D045H	1.13
45	D045F	1.11
45	D045L	1.05
45	D045V	1.05
45	D045Q	1.04
45	D045A	1.04
46	F046E	1.25
46	F046D	1.17

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
46	F046G	1.02
46	F046K	1.00
47	E047R	2.45
47	E047T	1.96
47	E047P	1.36
47	E047S	1.28
47	E047H	1.27
47	E047G	1.20
47	E047K	1.19
47	E047F	1.09
47	E047I	1.03
49	I049G	1.34
49	I049H	1.27
49	I049S	1.24
49	I049K	1.23
49	I049V	1.20
49	I049L	1.14
49	I049Y	1.07
49	I049R	1.05
49	I049E	1.02
49	I049M	1.01
50	E050L	1.19
50	E050M	1.18
50	E050A	1.12
51	E051V	1.47
51	E051A	1.28
51	E051G	1.22
51	E051T	1.18
51	E051L	1.11
51	E051I	1.07
53	L053H	5.05
53	L053Q	1.48

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
53	L053G	1.32
53	L053S	1.16
53	L053T	1.02
54	S054P	5.20
54	S054I	4.78
54	S054V	4.72
54	S054A	3.46
54	S054R	3.38
54	S054L	2.02
54	S054T	1.46
54	S054K	1.44
54	S054G	1.43
54	S054C	1.26
54	S054Q	1.03
55	A055G	1.69
55	A055T	1.69
57	T057S	1.63
57	T057R	1.61
57	T057V	1.28
57	T057I	1.19
59	N059W	1.13
59	N059R	1.09
59	N059T	1.07
59	N059S	1.06
59	N059Q	1.02
60	I060H	1.02
60	I060R	1.00
61	D061H	1.44
61	D061S	1.26
61	D061R	1.11
61	D061I	1.08
61	D061F	1.01

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
62	D062E	1.02
63	P063G	1.71
63	P063T	1.50
63	P063M	1.46
63	P063S	1.42
63	P063K	1.40
63	P063A	1.35
63	P063Y	1.35
63	P063W	1.35
63	P063V	1.31
63	P063R	1.31
63	P063F	1.25
63	P063L	1.15
63	P063Q	1.09
64	T064G	1.23
64	T064S	1.11
65	D065A	1.31
65	D065S	1.17
65	D065H	1.10
66	P066R	1.85
66	P066V	1.83
66	P066H	1.59
66	P066I	1.59
66	P066G	1.50
66	P066Q	1.46
66	P066T	1.41
66	P066S	1.39
66	P066Y	1.33
66	P066L	1.14
66	P066N	1.12
67	R067N	1.58
67	R067G	1.39

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
67	R067T	1.28
67	R067F	1.26
67	R067L	1.20
67	R067Q	1.16
67	R067W	1.07
67	R067E	1.04
67	R067P	1.01
68	L068E	1.44
68	L068W	1.21
68	L068I	1.13
68	L068G	1.09
68	L068V	1.09
68	L068H	1.05
68	L068T	1.03
69	N069V	1.99
69	N069K	1.72
69	N069R	1.49
69	N069I	1.47
69	N069H	1.36
69	N069T	1.35
69	N069L	1.30
69	N069S	1.21
69	N069G	1.20
69	N069Q	1.07
69	N069W	1.05
69	N069C	1.05
71	A071S	1.75
71	A071T	1.70
71	A071H	1.70
71	A071G	1.59
71	A071I	1.51
71	A071E	1.45

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
71	A071K	1.44
71	A071R	1.40
71	A071N	1.23
71	A071L	1.23
71	A071F	1.13
71	A071C	1.01
72	S072L	1.26
72	S072H	1.21
72	S072G	1.20
72	S072T	1.10
72	S072V	1.08
72	S072Y	1.07
73	Y073R	1.26
73	Y073Q	1.23
73	Y073S	1.17
73	Y073K	1.07
74	L074S	2.72
74	L074G	1.95
74	L074W	1.38
75	P075R	1.60
75	P075S	1.39
75	P075T	1.28
75	P075Q	1.21
75	P075G	1.16
75	P075H	1.05
75	P075W	1.04
76	S076P	1.23
77	C077T	1.12
77	C077V	1.05
77	C077G	1.01
78	L078G	4.98
78	L078H	4.82

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
78	L078E	3.01
78	L078N	2.68
78	L078T	1.87
78	L078Q	1.73
78	L078V	1.53
78	L078I	1.43
78	L078Y	1.39
79	A079H	1.93
79	A079L	1.80
79	A079I	1.59
79	A079M	1.50
79	A079N	1.48
79	A079Q	1.47
79	A079R	1.47
79	A079W	1.27
79	A079T	1.17
79	A079E	1.12
80	T080C	1.31
80	T080V	1.23
80	T080G	1.16
80	T080A	1.00
81	H081K	1.52
81	H081L	1.23
81	H081N	1.17
81	H081G	1.17
81	H081A	1.15
81	H081C	1.13
81	H081W	1.13
81	H081V	1.10
81	H081F	1.10
81	H081S	1.04
82	L082P	1.46
82	L082G	1.38
82	L082R	1.34
82	L082H	1.33
82	L082K	1.19
82	L082T	1.18
82	L082I	1.17
82	L082S	1.15
82	L082V	1.02
83	P083K	1.37
83	P083G	1.31
83	P083H	1.27
83	P083R	1.19
83	P083S	1.17
84	L084K	1.10
84	L084H	1.01
85	D085Q	3.09
85	D085R	2.38
85	D085S	2.28
85	D085H	1.55
85	D085N	1.54
85	D085G	1.41
85	D085T	1.33
85	D085E	1.12
85	D085F	1.01
86	L086A	1.38
86	L086C	1.16
86	L086G	1.15
88	I088H	1.20
88	I088T	1.03
88	I088G	1.01
90	M090T	1.27
90	M090I	1.13

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
90	M090V	1.08
90	M090S	1.06
90	M090L	1.02
91	L091G	1.21
91	L091T	1.06
92	G092V	1.49
92	G092S	1.26
93	T093Y	5.26
93	T093F	3.52
93	T093A	1.38
93	T093C	1.08
95	D095E	2.04
96	T096S	1.04
97	K097R	2.80
97	K097Q	1.14
98	A098L	2.22
98	A098H	2.09
98	A098I	2.05
98	A098Y	2.02
98	A098S	1.73
98	A098T	1.72
98	A098G	1.57
98	A098C	1.30
98	A098N	1.24
98	A098D	1.11
98	A098P	1.10
100	F100W	1.08
100	F100E	1.01
101	R101K	1.24
103	T103W	1.26
103	T103Y	1.19
103	T103G	1.11

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
103	T103K	1.09
103	T103I	1.08
103	T103L	1.05
104	P104H	2.84
104	P104T	2.70
104	P104G	2.67
104	P104V	2.59
104	P104S	2.48
104	P104I	2.43
104	P104W	2.05
104	P104C	1.95
104	P104E	1.84
104	P104F	1.79
104	P104N	1.62
104	P104R	1.62
104	P104Q	1.34
104	P104M	1.09
105	L105P	1.71
105	L105C	1.56
105	L105F	1.30
105	L105W	1.28
105	L105G	1.08
106	D106K	1.28
106	D106L	1.20
106	D106G	1.18
106	D106H	1.09
106	D106E	1.08
106	D106T	1.06
106	D106I	1.04
106	D106F	1.02
106	D106C	1.01
107	I107E	2.55

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
107	I107S	2.04
107	I107N	1.81
107	I107G	1.76
107	I107V	1.00
108	A108L	1.41
108	A108T	1.05
109	L109N	1.52
109	L109W	1.30
109	L109Q	1.18
109	L109Y	1.16
109	L109I	1.05
109	L109D	1.00
111	M111K	1.98
111	M111I	1.95
111	M111L	1.55
111	M111T	1.49
111	M111F	1.47
111	M111V	1.47
111	M111Y	1.43
111	M111S	1.03
112	S112L	1.03
112	S112H	1.00
113	V113L	1.50
113	V113H	1.34
113	V113K	1.19
113	V113R	1.13
113	V113Y	1.11
113	V113F	1.05
113	V113Q	1.03
115	V115W	1.23
115	V115T	1.15
115	V115L	1.12
115	V115G	1.09
115	V115I	1.05
115	V115Y	1.03
116	T116G	1.10
116	T116A	1.01
117	Q117H	2.33
117	Q117T	2.23
117	Q117Y	2.23
117	Q117W	2.16
117	Q117V	2.15
117	Q117G	2.08
117	Q117A	2.05
117	Q117S	1.95
117	Q117F	1.57
117	Q117R	1.56
117	Q117M	1.54
117	Q117E	1.15
118	V118Y	1.25
118	V118K	1.13
118	V118G	1.08
120	T120S	1.09
121	S121L	1.35
121	S121W	1.33
121	S121R	1.26
121	S121K	1.24
121	S121G	1.20
121	S121C	1.18
121	S121N	1.14
121	S121T	1.13
121	S121A	1.12
121	S121V	1.12
122	A122H	1.14

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
122	A122I	1.13
122	A122T	1.08
122	A122K	1.08
122	A122V	1.04
122	A122S	1.03
123	G123D	1.73
123	G123V	1.40
123	G123P	1.32
123	G123E	1.13
123	G123T	1.06
123	G123H	1.00
124	G124L	1.92
124	G124I	1.85
124	G124T	1.64
124	G124H	1.59
124	G124V	1.44
124	G124F	1.32
124	G124S	1.27
124	G124Y	1.23
124	G124R	1.14
124	G124Q	1.12
125	V125G	2.95
125	V125S	1.94
125	V125A	1.69
125	V125P	1.50
125	V125R	1.30
125	V125D	1.24
125	V125Y	1.08
125	V125I	1.01
126	G126T	1.58
126	G126P	1.17
126	G126L	1.17
127	T127H	1.57
127	T127V	1.07
127	T127I	1.06
127	T127S	1.05
128	T128L	1.06
128	T128K	1.06
130	P130T	1.19
130	P130H	1.17
130	P130K	1.16
130	P130G	1.16
130	P130S	1.16
130	P130V	1.15
130	P130W	1.15
130	P130I	1.12
130	P130L	1.12
130	P130R	1.11
130	P130F	1.08
130	P130E	1.00
131	A131L	1.83
131	A131R	1.76
131	A131H	1.72
131	A131G	1.66
131	A131W	1.61
131	A131V	1.59
131	A131P	1.52
131	A131Y	1.50
131	A131S	1.48
131	A131E	1.36
131	A131D	1.31
131	A131Q	1.29
132	P132Y	1.57
132	P132S	1.13

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Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
133	K133Y	1.12
133	K133L	1.05
133	K133H	1.02
134	V134G	1.71
134	V134T	1.25
134	V134N	1.18
134	V134S	1.16
134	V134L	1.13
134	V134I	1.12
136	V136T	1.13
137	V137M	1.22
137	V137L	1.09
137	V137T	1.08
137	V137A	1.07
137	V137G	1.02
138	S138I	1.15
138	S138G	1.05
140	P140A	1.90
140	P140T	1.74
140	P140S	1.31
141	P141L	2.32
141	P141I	2.29
141	P141H	2.07
141	P141V	1.96
141	P141T	1.84
141	P141S	1.70
141	P141R	1.65
141	P141G	1.64
141	P141Q	1.39
141	P141N	1.32
141	P141A	1.10
142	L142W	2.41
142	L142K	1.60
142	L142F	1.05
143	A143K	3.16
143	A143H	2.90
143	A143L	2.51
143	A143V	2.45
143	A143W	2.27
143	A143T	2.18
143	A143R	2.15
143	A143S	1.77
143	A143Q	1.74
143	A143F	1.56
143	A143P	1.53
143	A143G	1.48
143	A143D	1.45
143	A143E	1.43
143	A143C	1.39
143	A143N	1.30
144	P144Y	2.34
144	P144K	2.09
144	P144H	1.94
144	P144F	1.82
144	P144R	1.76
144	P144S	1.69
144	P144T	1.46
144	P144G	1.45
144	P144D	1.45
144	P144N	1.44
144	P144L	1.43
144	P144Q	1.37
144	P144M	1.24
144	P144A	1.09

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
145	M145L	1.72
145	M145F	1.49
145	M145R	1.15
145	M145W	1.15
145	M145C	1.02
145	M145T	1.01
147	H147A	1.28
147	H147S	1.26
147	H147T	1.20
147	H147P	1.12
147	H147E	1.11
148	P148V	2.43
148	P148K	1.79
148	P148L	1.64
148	P148A	1.64
148	P148R	1.51
148	P148T	1.50
148	P148Y	1.46
148	P148S	1.46
148	P148E	1.42
148	P148F	1.37
148	P148Q	1.33
148	P148D	1.03
150	F150L	1.29
150	F150E	1.23
151	Q151D	1.47
151	Q151R	1.36
151	Q151P	1.35
151	Q151A	1.29
151	Q151T	1.24
151	Q151M	1.24
151	Q151E	1.14

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
151	Q151K	1.07
151	Q151H	1.06
151	Q151S	1.05
151	Q151C	1.05
151	Q151Y	1.01
152	L152V	1.22
152	L152K	1.21
152	L152R	1.20
152	L152W	1.18
152	L152T	1.12
152	L152S	1.12
152	L152Y	1.09
152	L152H	1.09
152	L152G	1.08
152	L152E	1.08
152	L152Q	1.07
152	L152D	1.07
152	L152I	1.04
152	L152C	1.00
153	I153K	1.62
153	I153H	1.46
153	I153T	1.27
153	I153L	1.27
153	I153F	1.23
153	I153A	1.19
154	F154Y	1.32
155	E155T	1.49
155	E155R	1.47
155	E155L	1.31
155	E155Y	1.27
155	E155K	1.23
155	E155G	1.17

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
155	E155S	1.08
155	E155D	1.08
155	E155F	1.07
156	G156P	1.44
156	G156T	1.15
156	G156K	1.10
156	G156M	1.09
156	G156C	1.07
156	G156N	1.07
156	G156R	1.05
156	G156H	1.04
156	G156S	1.02
157	G157T	1.74
157	G157R	1.51
157	G157S	1.30
157	G157K	1.28
157	G157F	1.27
157	G157V	1.23
157	G157H	1.14
157	G157I	1.11
158	E158H	2.40
158	E158K	2.08
158	E158F	2.06
158	E158R	1.99
158	E158Y	1.77
158	E158W	1.77
158	E158L	1.59
158	E158S	1.57
158	E158V	1.52
158	E158Q	1.49
158	E158C	1.46
158	E158A	1.45
158	E158T	1.45
158	E158P	1.41
158	E158N	1.41
158	E158M	1.39
158	E158I	1.38
158	E158D	1.35
159	Q159R	1.15
159	Q159C	1.13
159	Q159S	1.10
159	Q159D	1.09
159	Q159A	1.08
159	Q159M	1.07
159	Q159P	1.06
159	Q159L	1.02
161	T161R	3.61
161	T161Y	2.40
161	T161H	1.82
161	T161W	1.41
161	T161I	1.40
161	T161V	1.27
161	T161L	1.25
161	T161Q	1.04
162	T162K	1.22
162	T162R	1.17
162	T162W	1.15
162	T162Y	1.03
162	T162H	1.02
163	E163L	1.50
163	E163Y	1.41
163	E163H	1.32
163	E163G	1.25
163	E163W	1.21

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
163	E163V	1.13
163	E163R	1.12
163	E163S	1.12
163	E163A	1.11
163	E163C	1.11
163	E163F	1.07
165	A165R	1.70
165	A165K	1.35
165	A165F	1.23
165	A165Q	1.21
165	A165V	1.21
165	A165Y	1.20
165	A165T	1.18
165	A165I	1.17
165	A165P	1.14
165	A165L	1.08
165	A165G	1.05
165	A165N	1.01
165	A165S	1.00
166	R166Y	1.29
166	R166L	1.27
166	R166I	1.26
166	R166W	1.25
166	R166H	1.20
166	R166T	1.19
166	R166V	1.17
166	R166K	1.17
166	R166S	1.16
166	R166G	1.15
167	V167T	1.13
167	V167I	1.08
167	V167Y	1.07
167	V167H	1.03
168	Y168G	1.89
168	Y168T	1.51
168	Y168V	1.19
169	S169Y	1.26
169	S169R	1.24
169	S169K	1.21
169	S169I	1.16
169	S169T	1.15
169	S169L	1.08
169	S169C	1.03
169	S169Q	1.02
170	A170K	1.71
170	A170G	1.59
170	A170I	1.59
170	A170S	1.47
170	A170F	1.44
170	A170T	1.40
170	A170E	1.28
170	A170D	1.27
170	A170N	1.21
170	A170V	1.20
170	A170C	1.15
170	A170Q	1.15
170	A170L	1.05
170	A170W	1.04
170	A170M	1.03
171	L171K	2.05
171	L171H	1.67
171	L171T	1.54
171	L171I	1.53
171	L171S	1.43

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
171	L171F	1.30
171	L171G	1.26
171	L171Y	1.20
171	L171V	1.02
172	A172I	1.70
172	A172S	1.59
172	A172W	1.43
172	A172G	1.41
172	A172V	1.40
172	A172T	1.25
172	A172L	1.20
172	A172C	1.20
173	S173Y	1.19
173	S173K	1.17
173	S173W	1.16
173	S173L	1.15
173	S173R	1.09
173	S173H	1.07
173	S173T	1.06
174	F174G	1.60
174	F174P	1.54
174	F174Q	1.42
174	F174C	1.32
174	F174S	1.16
174	F174L	1.05
175	M175T	2.21
175	M175G	2.04
175	M175V	1.93
175	M175L	1.61
175	M175Q	1.56
175	M175R	1.55
175	M175N	1.39

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./Var	Peracid formation relative to WT
175	M175W	1.25
176	K176W	1.19
176	K176T	1.04
176	K176Y	1.04
176	K176V	1.04
176	K176G	1.01
178	P178L	1.82
178	P178Y	1.38
178	P178K	1.34
178	P178W	1.14
178	P178G	1.09
179	F179L	1.15
179	F179Y	1.05
180	F180L	1.30
180	F180I	1.20
180	F180V	1.14
180	F180Y	1.12
180	F180W	1.11
180	F180K	1.08
180	F180T	1.01
181	D181A	1.35
181	D181K	1.33
181	D181Y	1.29
181	D181W	1.26
181	D181L	1.25
181	D181R	1.23
181	D181S	1.21
181	D181Q	1.14
181	D181E	1.10
181	D181G	1.09
181	D181C	1.09
181	D181P	1.03

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
181	D181T	1.02
182	A182T	1.14
184	S184Y	1.06
184	S184F	1.05
184	S184T	1.04
184	S184H	1.02
185	V185K	1.37
185	V185Y	1.37
185	V185W	1.36
185	V185H	1.30
185	V185L	1.23
185	V185R	1.15
185	V185G	1.12
185	V185T	1.11
185	V185S	1.09
185	V185I	1.07
185	V185F	1.02
186	I186G	1.86
186	I186T	1.51
186	I186A	1.46
186	I186S	1.39
186	I186V	1.28
186	I186L	1.17
186	I186F	1.01
187	S187K	1.45
187	S187Y	1.43
187	S187I	1.38
187	S187L	1.37
187	S187W	1.30
187	S187H	1.29
187	S187V	1.23
187	S187T	1.12
187	S187R	1.04
187	S187G	1.03
187	S187F	1.02
188	T188Y	1.48
188	T188V	1.22
188	T188S	1.16
188	T188I	1.13
188	T188H	1.11
188	T188R	1.01
189	D189L	1.30
189	D189H	1.25
189	D189W	1.09
190	G190W	1.88
190	G190K	1.01
191	V191Y	1.32
191	V191H	1.30
191	V191W	1.20
191	V191S	1.20
191	V191K	1.17
191	V191I	1.14
191	V191F	1.13
191	V191R	1.05
191	V191L	1.04
196	F196H	1.77
196	F196L	1.77
196	F196C	1.74
196	F196M	1.65
196	F196G	1.59
196	F196S	1.58
196	F196Y	1.41
196	F196V	1.40
196	F196I	1.32

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Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
196	F196W	1.01
197	T197L	1.21
198	E198R	1.82
198	E198I	1.80
198	E198V	1.60
198	E198W	1.59
198	E198L	1.57
198	E198P	1.52
198	E198Y	1.48
198	E198C	1.38
198	E198F	1.37
198	E198Q	1.28
198	E198T	1.25
198	E198N	1.24
198	E198M	1.18
198	E198S	1.06
199	A199C	1.77
199	A199K	1.72
199	A199E	1.56
199	A199L	1.38
199	A199T	1.33
199	A199R	1.33
199	A199V	1.32
199	A199D	1.31
199	A199H	1.27
199	A199Y	1.24
199	A199F	1.23
199	A199S	1.20
199	A199G	1.14
199	A199M	1.07
201	N201Y	1.29
201	N201F	1.16

Table 10-2. Variants with PAF Values Better Than Wild-Type

Pos	WT/Pos./ Var	Peracid formation relative to WT
201	N201G	1.08
202	R202W	1.97
202	R202F	1.89
202	R202E	1.69
202	R202H	1.64
202	R202T	1.55
202	R202S	1.49
202	R202A	1.48
202	R202C	1.44
202	R202M	1.43
202	R202L	1.43
202	R202G	1.39
202	R202I	1.33
203	D203L	2.42
203	D203R	2.23
203	D203I	1.99
203	D203W	1.99
203	D203F	1.92
203	D203H	1.84
203	D203C	1.78
203	D203S	1.66
203	D203V	1.66
203	D203G	1.63
203	D203Q	1.60
203	D203A	1.53
203	D203E	1.34
203	D203N	1.05

The following Table, provides variants with a PAF PI greater than 1.5.

Table 10-3. PAF PI > 1.5

Wild-Type Residue/Pos.	Variant Amino Acid(s)
A2	W
C7	H, I, K, Y
D10	K, L, W, Y
L12	C, Q
G15	A
E20	C, G, H, L, S, T, V, W
D21	K, W
G22	A
T25	G, S
E26	A, M
A29	G, R, V, W, Y
D31	L, W
Q40	G, I, K, L, N, R, S, T, W, Y
L42	H, K, W
A44	C, F, L, V
E47	R, T
L53	H
S54	A, I, L, P, R, V
A55	G, T
T57	R, S
P63	G
P66	H, I, R, V
R67	N
N69	K, V
A71	G, H, I, S, T
L74	G, S
P75	R
L78	E, G, H, N, Q, T, V
A79	H, I, L
H81	K
D85	H, N, Q, R, S
T93	F, Y
D95	E
K97	R
A98	G, H, I, L, S, T, Y
P104	C, E, F, G, H, I, N, R, S, T, V, W
L105	C, P
I107	E, G, N, S
L109	N
M111	I, K, L
V113	L
Q117	A, F, G, H, M, R, S, T, V, W, Y
G123	D, H, I, L, T
G124	I, L
V125	A, G, P, S
G126	T
T127	H
A131	G, H, L, P, R, V, W, Y
P132	Y
V134	G
P140	A, T,
P141	G, H, I, L, R, S, T, V
L142	K, W

Table 10-3. PAF PI > 1.5

Wild-Type Residue/Pos.	Variant Amino Acid(s)
A143	F, H, K, L, P, Q, R, S, T, V, W
P144	F, H, K, R, S, Y
M145	L
P148	A, K, L, R, T, V
I153	K
G157	R, T
E158	F, H, K, L, R, S, V, W, Y
T161	H, R, Y
A165	T
Y168	G, T
A170	G, I, K
L171	H, I, K, T
A172	I, S
F174	G, P
M175	G, L, Q, R, T, V
P178	L
F196	C, G, H, L, M, S
G190	W
E198	I, L, P, R, V, W
A199	C, E, K
R202	E, F, H, T, W
D203	A, C, F, G, H, I, L, Q, R, S, V, W
V206	E, F, G, H, K, R, S,
A209	K
E210	H, K, S, T, V, W
Q211	K
V212	W

Table 10-4 provides variants with PAF PI values greater than 2.0.

Table 10-4. Variants with PAF PI > 2.0

Wild-Type Residue/Pos.	Amino Acid Variant(s)
C7	K, Y
D10	K. L, W
L12	C, Q
E20	G, H, L, S, T, V, W
E26	M
Q40	I, K, L, T, W
L42	K, W
A44	F, V
E47	R
L53	H
S54	A, I, L, P, R, V
L74	S
L78	E, G, H, N
D85	Q, R, S
T93	F, Y
D95	E
K97	R
A98	H, I, L, Y
P104	G, H, I, S, T. V, W
I107	E, S
Q117	A, G, H, T, V, W, Y
V125	G
P141	H, I, L
L142	W
A143	H, K, L, R, T, V, W
P144	K, Y
P148	V
E158	F, H, K
T161	R, Y
L171	K
M175	G, T
D203	L, R
V206	E, F, K
E210	T

The following Table provides PAD assay results for various variants.

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
1	M001A	A	<0.01
1	M001E	E	<0.01
1	M001F	F	<0.01
1	M001G	G	<0.01
1	M001K	K	<0.01
1	M001N	N	<0.01
1	M001P	P	<0.01
1	M001R	R	<0.01
1	M001S	S	<0.01
1	M001T	T	<0.01
1	M001W	W	<0.01
1	M001V	V	0.944944
3	K003V	V	0.835476
4	R004L	L	<0.01
4	R004V	V	0.079216
4	R004I	I	0.153122
4	R004W	W	0.484006
4	R004G	G	0.78952
4	R004S	S	0.907174
4	R004E	E	0.970668
4	R004Y	Y	0.983327
4	R004H	H	0.986096
4	R004Q	Q	0.98766
4	R004T	T	0.999841
5	I005G	G	<0.01
5	I005N	N	<0.01
5	I005P	P	<0.01
5	I005R	R	<0.01
5	I005W	W	<0.01
5	I005F	F	0.15045
5	I005S	S	0.367738
5	I005H	H	0.626022
5	I005T	T	0.7212
5	I005V	V	0.917243
6	L006S	S	<0.01
6	L006K	K	<0.01
6	L006G	G	<0.01
6	L006H	H	<0.01
6	L006R	R	<0.01
6	L006W	W	<0.01
6	L006E	E	<0.01
6	L006Q	Q	<0.01
6	L006V	V	0.352616
6	L006T	T	0.354148
6	L006I	I	0.819654
7	C007S	S	<0.01
7	C007R	R	<0.01
7	C007L	L	<0.01
7	C007P	P	<0.01
7	C007T	T	<0.01
7	C007W	W	<0.01
7	C007Y	Y	0.544454

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
7	C007M	M	0.678238
7	C007G	G	0.686018
10	D010W	W	<0.01
10	D010K	K	<0.01
10	D010Y	Y	<0.01
10	D010T	T	<0.01
10	D010I	I	<0.01
10	D010V	V	<0.01
10	D010S	S	<0.01
10	D010G	G	<0.01
10	D010R	R	<0.01
10	D010A	A	<0.01
10	D010M	M	<0.01
10	D010N	N	<0.01
10	D010P	P	<0.01
10	D010E	E	0.147899
11	S011T	T	<0.01
11	S011V	V	<0.01
11	S011D	D	<0.01
11	S011E	E	<0.01
11	S011F	F	<0.01
11	S011G	G	<0.01
11	S011L	L	<0.01
11	S011Q	Q	<0.01
11	S011R	R	<0.01
11	S011H	H	0.332012
11	S011K	K	0.399168
11	S011A	A	0.528328
11	S011I	I	0.562735
12	L012V	V	<0.01
12	L012S	S	<0.01
12	L012G	G	<0.01
12	L012R	R	<0.01
12	L012D	D	<0.01
12	L012P	P	<0.01
12	L012W	W	<0.0162738575856614
12	L012T	T	0.064264
12	L012A	A	0.074567
12	L012K	K	0.134919
12	L012H	H	0.164894
12	L012F	F	0.171369
12	L012Q	Q	0.219754
12	L012C	C	0.221492
12	L012N	N	0.655242
13	T013F	F	<0.01
13	T013R	R	<0.01
13	T013W	W	<0.01
13	T013Q	Q	0.508867
13	T013V	V	0.625148
13	T013S	S	0.682494
13	T013G	G	0.768701
14	W014I	I	<0.01
14	W014S	S	<0.01
14	W014G	G	<0.01
14	W014K	K	<0.01
14	W014V	V	<0.01
14	W014L	L	<0.01

Table 10-5. PAD Assay Results			
Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
14	W014T	T	<0.01
14	W014R	R	<0.01
14	W014N	N	<0.01
14	W014P	P	<0.01
14	W014E	E	0.150043
14	W014F	F	0.218073
14	W014A	A	0.271277
14	W014Y	Y	0.64896
14	W014W	W	0.989643
15	G015C	C	<0.01
15	G015N	N	<0.01
15	G015D	D	<0.01
15	G015E	E	<0.01
15	G015H	H	<0.01
15	G015K	K	<0.01
15	G015L	L	<0.01
15	G015P	P	<0.01
15	G015R	R	<0.01
15	G015Y	Y	<0.01
15	G015A	A	0.614319
15	G015S	S	0.631317
16	W016S	S	<0.01
16	W016G	G	<0.01
16	W016H	H	<0.01
16	W016N	N	<0.01
16	W016R	R	<0.01
16	W016T	T	<0.01
16	W016P	P	0.150383
16	W016Q	Q	0.312038

Table 10-5. PAD Assay Results			
Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
16	W016M	M	0.370155
16	W016A	A	0.553088
16	W016D	D	0.569713
16	W016E	E	0.647375
16	W016V	V	0.875327
17	V017A	A	0.675391
17	V017E	E	0.749717
17	V017G	G	0.838345
17	V017K	K	0.844479
17	V017F	F	0.847091
17	V017T	T	0.861827
17	V017Y	Y	0.876678
17	V017R	R	0.936013
17	V017P	P	0.956795
17	V017I	I	0.993337
17	V017L	L	0.996217
18	P018A	A	<0.01
18	P018M	M	<0.01
18	P018S	S	0.066689
19	V019P	P	<0.01
19	V019M	M	0.117174
19	V019R	R	0.343385
19	V019Q	Q	0.395965
19	V019A	A	0.554598
19	V019G	G	0.55596
19	V019S	S	0.573928
19	V019E	E	0.620236
19	V019Y	Y	0.696626
19	V019D	D	0.785756

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
19	V019L	L	0.910961
19	V019K	K	0.965611
21	D021V	V	<0.01
21	D021P	P	0.534939
21	D021S	S	0.689672
21	D021E	E	0.864655
21	D021F	F	0.876655
21	D021W	W	0.894205
21	D021L	L	0.971454
22	G022K	K	<0.01
22	G022W	W	0.231005
22	G022R	R	0.563069
22	G022V	V	0.850851
22	G022S	S	0.981692
23	A023R	R	0.283095
23	A023S	S	0.335177
23	A023G	G	0.350575
23	A023F	F	0.438047
23	A023V	V	0.598414
23	A023Q	Q	0.732052
23	A023P	P	0.733451
23	A023W	W	0.801206
23	A023M	M	0.946802
23	A023Y	Y	0.962455
24	P024S	S	0.614708
24	P024Q	Q	0.652848
24	P024T	T	0.663925
24	P024A	A	0.681992
24	P024G	G	0.755229
24	P024I	I	0.853247
24	P024R	R	0.907892
24	P024H	H	0.969695
25	T025P	P	<0.01
25	T025H	H	<0.01
25	T025L	L	<0.01
25	T025R	R	<0.01
25	T025M	M	<0.01
25	T025E	E	<0.01
25	T025D	D	<0.01
25	T025K	K	0.133406
25	T025W	W	0.144315
25	T025I	I	0.350917
25	T025G	G	0.426214
25	T025C	C	0.509792
25	T025V	V	0.514769
25	T025S	S	0.576256
25	T025A	A	0.863346
26	E026S	S	0.280953
26	E026T	T	0.39705
26	E026W	W	0.471182
26	E026N	N	0.47572
26	E026R	R	0.813632
26	E026G	G	0.869755
26	E026C	C	0.939981
26	E026V	V	0.966156
26	E026P	P	0.993535
27	R027W	W	<0.01
27	R027T	T	<0.01497896

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
			77895526
27	R027P	P	0.483512
27	R027C	C	0.58498
27	R027S	S	0.686775
27	R027G	G	0.836174
27	R027E	E	0.925988
27	R027V	V	0.943209
28	F028G	G	<0.01
28	F028H	H	<0.01
28	F028I	I	<0.01
28	F028R	R	<0.01
28	F028P	P	0.385272
28	F028V	V	0.531941
28	F028S	S	0.696363
29	A029V	V	0.43718
29	A029T	T	0.467508
29	A029S	S	0.546873
29	A029Y	Y	0.593264
29	A029P	P	0.622623
29	A029R	R	0.728312
29	A029W	W	0.738583
29	A029M	M	0.768108
29	A029G	G	0.802278
29	A029E	E	0.844095
29	A029D	D	0.996225
30	P030M	M	0.78893
30	P030Q	Q	0.905135
30	P030A	A	0.918048
31	D031E	E	0.882779

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
27	R027P	P	0.483512
27	R027C	C	0.58498
27	R027S	S	0.686775
27	R027G	G	0.836174
27	R027E	E	0.925988
27	R027V	V	0.943209
28	F028G	G	<0.01
28	F028H	H	<0.01
28	F028I	I	<0.01
28	F028R	R	<0.01
28	F028P	P	0.385272
28	F028V	V	0.531941
28	F028S	S	0.696363
29	A029V	V	0.43718
29	A029T	T	0.467508
29	A029S	S	0.546873
29	A029Y	Y	0.593264
29	A029P	P	0.622623
29	A029R	R	0.728312
29	A029W	W	0.738583
29	A029M	M	0.768108
29	A029G	G	0.802278
29	A029E	E	0.844095
29	A029D	D	0.996225
30	P030M	M	0.78893
30	P030Q	Q	0.905135
30	P030A	A	0.918048
31	D031E	E	0.882779

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
32	V032P	P	<0.01
32	V032R	R	0.715259
33	R033D	D	<0.01
33	R033E	E	<0.01
33	R033H	H	<0.01
33	R033P	P	<0.01
33	R033W	W	<0.01
33	R033V	V	0.935183
34	W034R	R	<0.01
34	W034E	E	<0.01
34	W034K	K	<0.01
34	W034Q	Q	0.041311
34	W034S	S	0.079486
34	W034T	T	0.153641
34	W034V	V	0.72591
34	W034G	G	0.880049
34	W034I	I	0.93831
35	T035Q	Q	<0.01
35	T035N	N	<0.01
35	T035R	R	<0.01
35	T035K	K	<0.01
35	T035L	L	<0.01
35	T035P	P	<0.01
35	T035W	W	<0.01
35	T035Y	Y	<0.01
35	T035V	V	0.344374
36	G036P	P	<0.01
36	G036S	S	0.25722
36	G036T	T	0.326076

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
36	G036V	V	0.375828
36	G036M	M	0.536338
36	G036N	N	0.557724
36	G036W	W	0.682701
36	G036Q	Q	0.712029
36	G036R	R	0.897684
38	L038K	K	<0.01
38	L038G	G	<0.01
38	L038E	E	<0.01
38	L038P	P	<0.01
38	L038Q	Q	<0.01
38	L038R	R	<0.01
38	L038W	W	<0.01
40	Q040P	P	<0.01
41	Q041V	V	<0.01
41	Q041S	S	0.222419
41	Q041P	P	0.662368
41	Q041Y	Y	0.701492
41	Q041W	W	0.878483
42	L042W	W	<0.01
42	L042H	H	<0.01
42	L042T	T	<0.01
42	L042D	D	<0.01
42	L042Q	Q	0.280991
42	L042S	S	0.450557
42	L042R	R	0.64188
42	L042I	I	0.658658
42	L042V	V	0.725221
42	L042M	M	0.73687

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
42	L042G	G	0.759964
43	G043S	S	0.233902
43	G043P	P	0.310899
43	G043V	V	0.332639
43	G043Q	Q	0.475759
43	G043R	R	0.585481
43	G043C	C	0.725373
43	G043I	I	0.766408
43	G043K	K	0.856798
43	G043M	M	0.877674
43	G043Y	Y	0.944457
43	G043H	H	0.957156
44	A044S	S	<0.01
44	A044Y	Y	<0.01
44	A044T	T	<0.01
44	A044R	R	<0.01
44	A044D	D	<0.01
44	A044H	H	<0.01
44	A044P	P	<0.01
44	A044E	E	0.028463
44	A044V	V	0.504951
44	A044F	F	0.803847
44	A044W	W	0.847767
44	A044M	M	0.975188
44	A044L	L	0.99381
45	D045S	S	0.382964
45	D045T	T	0.438291
45	D045R	R	0.492492
45	D045V	V	0.500129
45	D045P	P	0.531241
45	D045Q	Q	0.568687
45	D045W	W	0.582004
45	D045H	H	0.779564
45	D045L	L	0.781626
45	D045M	M	0.78286
45	D045G	G	0.839279
45	D045A	A	0.841569
45	D045C	C	0.844725
45	D045K	K	0.867296
46	F046H	H	<0.01
46	F046T	T	0.429962
46	F046W	W	0.633171
46	F046S	S	0.656356
46	F046V	V	0.786355
46	F046I	I	0.882982
46	F046G	G	0.944614
47	E047P	P	0.357072
47	E047R	R	0.620501
47	E047N	N	0.627512
47	E047S	S	0.628088
47	E047M	M	0.703134
47	E047A	A	0.757492
47	E047F	F	0.763159
47	E047C	C	0.772744
47	E047T	T	0.837562
47	E047D	D	0.975388
47	E047H	H	0.99217
48	V048R	R	<0.01

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
48	V048W	W	<0.01
48	V048S	S	0.423613
48	V048G	G	0.873544
48	V048N	N	0.980906
48	V048E	E	0.987222
49	I049P	P	0.161279
49	I049R	R	0.29139
49	I049W	W	0.676641
49	I049H	H	0.740799
49	I049S	S	0.789362
49	I049E	E	0.876247
49	I049V	V	0.972022
50	E050R	R	<0.01
50	E050W	W	0.14091
50	E050V	V	0.425221
50	E050I	I	0.575369
50	E050S	S	0.645021
50	E050Q	Q	0.906441
50	E050L	L	0.967983
51	E051R	R	<0.01
51	E051P	P	<0.01
51	E051I	I	0.044391
51	E051W	W	0.165053
51	E051V	V	0.367755
51	E051Q	Q	0.761883
51	E051L	L	0.927544
52	G052H	H	<0.01
52	G052S	S	<0.01
52	G052V	V	<0.01
52	G052T	T	<0.01
52	G052M	M	<0.01
52	G052F	F	<0.01
52	G052I	I	0.069022
52	G052P	P	0.242545
52	G052L	L	0.244397
52	G052Q	Q	0.283827
52	G052R	R	0.349923
52	G052E	E	0.549067
52	G052A	A	0.793929
53	L053R	R	<0.01
53	L053W	W	<0.01
53	L053P	P	<0.01
53	L053D	D	<0.01328259968325
53	L053E	E	0.191623
53	L053K	K	0.237686
53	L053S	S	0.260431
53	L053G	G	0.32712
53	L053V	V	0.652864
53	L053I	I	0.659806
53	L053Q	Q	0.717093
53	L053T	T	0.842042
54	S054F	F	<0.01
54	S054W	W	<0.01
54	S054H	H	<0.01
54	S054K	K	0.083519
54	S054I	I	0.116295
54	S054Y	Y	0.124722

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
54	S054G	G	0.170484
54	S054L	L	0.258821
54	S054V	V	0.285755
54	S054E	E	0.296919
54	S054T	T	0.329279
54	S054R	R	0.354857
54	S054M	M	0.482666
54	S054Q	Q	0.531633
54	S054D	D	0.647787
54	S054C	C	0.87772
55	A055V	V	<0.01
55	A055I	I	<0.01
55	A055P	P	<0.01
55	A055W	W	<0.01
55	A055Y	Y	0.176777
55	A055R	R	0.245648
55	A055T	T	0.415054
55	A055G	G	0.731513
55	A055L	L	0.866592
55	A055S	S	0.866756
55	A055H	H	0.921909
56	R056C	C	<0.01
56	R056G	G	<0.01
56	R056T	T	<0.01
56	R056E	E	<0.01
56	R056H	H	<0.01
56	R056K	K	<0.01
56	R056P	P	<0.01
56	R056Q	Q	<0.01
56	R056W	W	<0.01
56	R056Y	Y	<0.01
56	R056S	S	0.123501
56	R056L	L	0.237933
56	R056N	N	0.267811
56	R056A	A	0.68802
57	T057R	R	<0.01
57	T057P	P	<0.01
57	T057W	W	<0.01
57	T057N	N	0.245605
57	T057C	C	0.398001
57	T057Y	Y	0.551709
57	T057H	H	0.605386
57	T057A	A	0.651879
57	T057L	L	0.762087
57	T057V	V	0.86913
57	T057I	I	0.870692
58	T058E	E	<0.01
58	T058G	G	<0.01
58	T058K	K	<0.01
58	T058P	P	<0.01
58	T058R	R	<0.01
58	T058W	W	<0.01
58	T058Y	Y	<0.01
58	T058M	M	0.026886
58	T058A	A	0.361258
58	T058V	V	0.955494
58	T058S	S	0.964758
59	N059R	R	<0.01

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
59	N059M	M	<0.01
59	N059P	P	<0.01
59	N059Q	Q	0.165409
59	N059T	T	0.501362
59	N059S	S	0.651989
59	N059K	K	0.731191
59	N059E	E	0.879272
59	N059V	V	0.887341
59	N059G	G	0.890006
59	N059F	F	0.911279
59	N059A	A	0.929578
59	N059Y	Y	0.99189
59	N059C	C	0.99959
60	I060P	P	0.318965
60	I060D	D	0.660273
60	I060C	C	0.668516
60	I060M	M	0.682237
60	I060A	A	0.788799
60	I060R	R	0.809655
60	I060L	L	0.913226
60	I060E	E	0.923286
60	I060K	K	0.959958
60	I060S	S	0.999829
61	D061F	F	0.698154
61	D061A	A	0.708121
61	D061C	C	0.848446
61	D061Y	Y	0.948278
61	D061V	V	0.968066
61	D061N	N	0.999276
62	D062T	T	<0.01
62	D062I	I	<0.01
62	D062V	V	<0.01
62	D062H	H	<0.01
62	D062W	W	<0.01
62	D062S	S	<0.01
62	D062L	L	<0.01
62	D062G	G	<0.01
62	D062R	R	<0.01
62	D062M	M	<0.01
62	D062P	P	<0.01
62	D062Q	Q	<0.01
62	D062A	A	0.113753
62	D062C	C	0.490736
62	D062E	E	0.602369
63	P063A	A	0.598416
63	P063R	R	0.801911
63	P063S	S	0.898408
63	P063M	M	0.908904
63	P063F	F	0.925844
63	P063Y	Y	0.948378
64	T064R	R	0.106209
64	T064D	D	0.640095
64	T064W	W	0.691185
64	T064Q	Q	0.865168
64	T064C	C	0.876862
64	T064P	P	0.936023
64	T064H	H	0.960718
64	T064N	N	0.983933

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
64	T064S	S	0.987972
65	D065V	V	0.199467
65	D065R	R	0.215599
65	D065H	H	0.398178
65	D065Y	Y	0.42301
65	D065P	P	0.423122
65	D065S	S	0.468174
65	D065W	W	0.50219
65	D065T	T	0.5039
65	D065G	G	0.51655
65	D065I	I	0.617391
65	D065A	A	0.723321
66	P066N	N	0.381273
66	P066Q	Q	0.422614
66	P066G	G	0.444859
66	P066R	R	0.508806
66	P066C	C	0.523524
66	P066A	A	0.563865
66	P066F	F	0.672865
66	P066Y	Y	0.699931
66	P066D	D	0.718749
66	P066I	I	0.844376
66	P066V	V	0.89302
66	P066H	H	0.947771
66	P066L	L	0.987271
67	R067F	F	<0.0149736260903786
67	R067W	W	<0.0171329732205367
67	R067P	P	0.036575
67	R067E	E	0.113415
67	R067V	V	0.1203
67	R067Q	Q	0.126838
67	R067L	L	0.156654
67	R067A	A	0.215271
67	R067T	T	0.315404
67	R067N	N	0.333066
67	R067G	G	0.40823
67	R067K	K	0.986487
68	L068G	G	<0.01
68	L068A	A	<0.01
68	L068M	M	0.02834
68	L068C	C	0.05996
68	L068S	S	0.071622
68	L068N	N	0.100981
68	L068E	E	0.131505
68	L068H	H	0.222734
68	L068Q	Q	0.254448
68	L068F	F	0.254797
68	L068T	T	0.324904
68	L068P	P	0.35297
68	L068D	D	0.443469
68	L068Y	Y	0.447862
68	L068R	R	0.465293
68	L068V	V	0.507389
68	L068W	W	0.561612
68	L068I	I	0.727312
69	N069Y	Y	0.173925
69	N069W	W	0.55063

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
69	N069P	P	0.591783
69	N069R	R	0.828172
69	N069G	G	0.976332
70	G070M	M	<0.01
70	G070T	T	<0.01
70	G070P	P	<0.01
70	G070V	V	<0.01
70	G070C	C	<0.01
70	G070R	R	<0.01
70	G070Y	Y	<0.01
70	G070K	K	<0.01
70	G070N	N	<0.01
70	G070Q	Q	<0.01
70	G070F	F	<0.01
70	G070I	I	0.270463
70	G070E	E	0.33356
70	G070S	S	0.638917
71	A071P	P	<0.01
71	A071N	N	0.613838
71	A071D	D	0.646588
71	A071G	G	0.675895
71	A071S	S	0.693249
71	A071R	R	0.771492
71	A071H	H	0.781953
71	A071I	I	0.786894
71	A071T	T	0.79386
71	A071E	E	0.809505
71	A071L	L	0.838126
71	A071F	F	0.985677
71	A071C	C	0.993683
72	S072Y	Y	0.069096
72	S072W	W	0.339835
72	S072P	P	0.555612
72	S072Q	Q	0.655328
72	S072L	L	0.703483
72	S072R	R	0.742354
72	S072D	D	0.800127
72	S072V	V	0.82827
72	S072E	E	0.930527
72	S072T	T	0.973836
73	Y073P	P	<0.01
73	Y073R	R	0.262561
73	Y073L	L	0.497588
73	Y073G	G	0.509699
73	Y073H	H	0.515737
73	Y073I	I	0.641914
73	Y073S	S	0.676285
73	Y073V	V	0.73535
73	Y073N	N	0.758401
73	Y073D	D	0.803442
73	Y073Q	Q	0.866092
73	Y073K	K	0.944166
76	S076W	W	<0.01
76	S076Y	Y	0.177113
76	S076F	F	0.461095
76	S076Q	Q	0.900789
77	C077Y	Y	<0.01
77	C077R	R	<0.01

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Table 10-5. PAD Assay Results			
Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
77	C077W	W	<0.01
77	C077F	F	<0.01
77	C077N	N	<0.01
77	C077P	P	<0.01
77	C077G	G	0.181068
77	C077L	L	0.734708
77	C077S	S	0.764136
77	C077V	V	0.802259
77	C077A	A	0.912937
78	L078E	E	<0.01
78	L078N	N	<0.01
78	L078A	A	<0.01
78	L078P	P	<0.01
78	L078R	R	<0.01
78	L078S	S	<0.01
78	L078M	M	0.477538
78	L078Q	Q	0.519566
78	L078C	C	0.779536
78	L078Y	Y	0.809511
78	L078V	V	0.827484
79	A079H	H	<0.01
79	A079F	F	<0.01
79	A079V	V	<0.01
79	A079C	C	0.026887
79	A079Q	Q	0.268704
79	A079E	E	0.272158
79	A079N	N	0.281684
79	A079M	M	0.284387
79	A079R	R	0.321618

Table 10-5. PAD Assay Results			
Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
79	A079W	W	0.530746
79	A079T	T	0.598368
79	A079I	I	0.673986
79	A079S	S	0.779628
79	A079G	G	0.915372
79	A079P	P	0.94147
79	A079L	L	0.958677
80	T080W	W	<0.01
80	T080L	L	<0.01
80	T080K	K	<0.01
80	T080R	R	<0.01
80	T080E	E	<0.01
80	T080P	P	<0.01
80	T080H	H	0.049717
80	T080Y	Y	0.107973
80	T080I	I	0.146188
80	T080N	N	0.529867
82	L082R	R	<0.01
82	L082S	S	<0.01
82	L082W	W	<0.01
82	L082V	V	0.187819
82	L082G	G	0.310823
82	L082T	T	0.377413
82	L082H	H	0.468806
82	L082I	I	0.508005
82	L082K	K	0.508537
82	L082P	P	0.516154
82	L082A	A	0.976228
83	P083T	T	<0.01

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
83	P083V	V	0.186837
83	P083L	L	0.211018
83	P083H	H	0.611439
83	P083W	W	0.621496
83	P083G	G	0.677444
83	P083S	S	0.789585
83	P083Q	Q	0.818267
83	P083D	D	0.831344
83	P083F	F	0.99445
84	L084W	W	<0.01
84	L084V	V	0.416576
84	L084P	P	0.43025
84	L084T	T	0.438956
84	L084A	A	0.453182
84	L084Q	Q	0.516002
84	L084S	S	0.550862
84	L084R	R	0.565943
84	L084N	N	0.665228
84	L084K	K	0.79008
84	L084D	D	0.85276
84	L084I	I	0.870124
84	L084H	H	0.993217
85	D085I	I	0.100248
85	D085L	L	0.241561
85	D085V	V	0.25268
85	D085W	W	0.341677
85	D085P	P	0.543807
85	D085Y	Y	0.554364
85	D085S	S	0.675803
85	D085T	T	0.708548
85	D085N	N	0.781957
85	D085Q	Q	0.988545
86	L086H	H	<0.01
86	L086S	S	<0.01
86	L086R	R	<0.01
86	L086E	E	<0.01
86	L086F	F	<0.01
86	L086Q	Q	<0.01
86	L086W	W	0.077717
86	L086V	V	0.120133
86	L086T	T	0.284184
86	L086G	G	0.696393
86	L086Y	Y	0.815121
86	L086P	P	0.987233
87	V087S	S	<0.01
87	V087G	G	<0.01
87	V087Y	Y	<0.01
87	V087R	R	<0.01
87	V087K	K	<0.01
87	V087D	D	<0.01
87	V087F	F	0.103908
87	V087T	T	0.147618
87	V087A	A	0.16806
87	V087M	M	0.751854
89	I089H	H	<0.01
89	I089S	S	<0.01
89	I089G	G	<0.01
89	I089W	W	<0.01

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
89	I089Q	Q	<0.01
89	I089D	D	<0.01
89	I089E	E	<0.01
89	I089R	R	<0.01
89	I089F	F	0.745747
89	I089V	V	0.820031
89	I089T	T	0.900425
94	N094L	L	<0.01
94	N094T	T	<0.01
94	N094V	V	<0.01
94	N094H	H	<0.01
94	N094R	R	<0.01
94	N094W	W	<0.01
94	N094M	M	0.031458
94	N094C	C	0.072751
94	N094Y	Y	0.123924
94	N094G	G	0.532837
94	N094A	A	0.74316
94	N094P	P	0.789771
94	N094S	S	0.877698
95	D095A	A	<0.01
95	D095C	C	<0.01
95	D095G	G	<0.01
95	D095H	H	<0.01
95	D095K	K	<0.01
95	D095L	L	<0.01
95	D095N	N	<0.01
95	D095Q	Q	<0.01
95	D095R	R	<0.01
95	D095S	S	<0.01
95	D095T	T	<0.01
95	D095V	V	<0.01
95	D095W	W	<0.01
95	D095Y	Y	<0.01
95	D095E	E	0.754335
96	T096I	I	<0.01
96	T096W	W	<0.01
96	T096Y	Y	<0.01
96	T096R	R	0.136108
96	T096V	V	0.58611
96	T096S	S	0.786547
96	T096P	P	0.885134
97	K097Q	Q	<0.01
97	K097G	G	<0.01
97	K097I	I	<0.01
97	K097W	W	<0.01
97	K097L	L	<0.01
97	K097V	V	<0.01
97	K097Y	Y	<0.01
97	K097S	S	<0.01
97	K097T	T	<0.01
97	K097D	D	<0.01
97	K097M	M	0.216645
97	K097A	A	0.227977
97	K097P	P	0.26585
97	K097R	R	0.587184
99	Y099R	R	0.291941
99	Y099V	V	0.311502

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
99	Y099S	S	0.367181
99	Y099W	W	0.566038
99	Y099H	H	0.591623
99	Y099I	I	0.60574
99	Y099G	G	0.700083
99	Y099P	P	0.813989
99	Y099A	A	0.822549
99	Y099L	L	0.856204
100	F100W	W	<0.01
100	F100K	K	<0.01
100	F100D	D	<0.01
100	F100E	E	0.152427
100	F100S	S	0.852784
101	R101W	W	<0.01
101	R101K	K	0.068708
101	R101Q	Q	0.107171
101	R101V	V	0.442582
101	R101D	D	0.800722
101	R101Y	Y	0.803109
101	R101P	P	0.855496
101	R101N	N	0.918012
101	R101C	C	0.946306
101	R101I	I	0.955711
101	R101F	F	0.965422
102	R102W	W	<0.01
102	R102F	F	0.226881
102	R102G	G	0.270733
102	R102C	C	0.363718
102	R102V	V	0.60605
102	R102D	D	0.684234
102	R102P	P	0.894709
102	R102S	S	0.960127
103	T103W	W	<0.01
103	T103Y	Y	<0.01
103	T103G	G	<0.01
103	T103K	K	<0.01
103	T103I	I	<0.01
103	T103L	L	<0.01
103	T103H	H	<0.01
103	T103A	A	<0.01
103	T103V	V	<0.01
103	T103S	S	<0.01
103	T103C	C	<0.01
103	T103R	R	<0.01
103	T103N	N	<0.01
103	T103F	F	<0.01
103	T103P	P	<0.01
104	P104R	R	<0.01
104	P104A	A	<0.01
104	P104L	L	<0.01
104	P104W	W	0.232802
104	P104T	T	0.333526
104	P104S	S	0.529113
104	P104Q	Q	0.847699
104	P104F	F	0.863543
104	P104G	G	0.984538
105	L105V	V	<0.01
105	L105A	A	<0.01

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
105	L105M	M	<0.01
105	L105E	E	0.528458
105	L105S	S	0.609931
105	L105Y	Y	0.620029
105	L105T	T	0.638962
105	L105P	P	0.902642
106	D106R	R	0.559786
106	D106Q	Q	0.617485
106	D106P	P	0.632087
106	D106N	N	0.642667
106	D106M	M	0.855673
106	D106I	I	0.915931
106	D106L	L	0.99561
107	I107E	E	<0.01
107	I107G	G	<0.01
107	I107F	F	<0.01
107	I107Q	Q	<0.01
107	I107R	R	<0.01
107	I107H	H	<0.01
107	I107W	W	<0.01
107	I107P	P	0.318743
107	I107Y	Y	0.524182
107	I107A	A	0.795478
107	I107N	N	0.929935
107	I107V	V	0.96863
108	A108D	D	<0.01
108	A108F	F	<0.01
108	A108H	H	<0.01
108	A108I	I	<0.01

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
108	A108N	N	<0.01
108	A108P	P	<0.01
108	A108R	R	<0.01
108	A108E	E	0.60726
108	A108Q	Q	0.734472
108	A108T	T	0.865471
108	A108V	V	0.950481
109	L109W	W	<0.01
109	L109D	D	0.106206
109	L109I	I	0.144257
109	L109E	E	0.194168
109	L109R	R	0.210346
109	L109H	H	0.220153
109	L109Q	Q	0.222755
109	L109F	F	0.317718
109	L109A	A	0.323528
109	L109S	S	0.378623
109	L109P	P	0.434661
109	L109G	G	0.51022
109	L109V	V	0.539733
109	L109M	M	0.628881
109	L109N	N	0.658369
109	L109T	T	0.79132
109	L109Y	Y	0.825105
110	G110T	T	<0.01
110	G110L	L	<0.01
110	G110W	W	<0.01
110	G110Y	Y	<0.01
110	G110P	P	0.224284

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
110	G110I	I	0.232219
110	G110S	S	0.30218
110	G110Q	Q	0.343918
110	G110R	R	0.476072
110	G110H	H	0.73456
110	G110N	N	0.770851
110	G110M	M	0.816422
111	M111R	R	<0.01
111	M111S	S	0.139078
111	M111H	H	0.192733
111	M111G	G	0.315165
111	M111P	P	0.566892
111	M111E	E	0.668985
111	M111L	L	0.67115
111	M111K	K	0.706165
111	M111T	T	0.763332
111	M111F	F	0.776934
111	M111D	D	0.78777
111	M111V	V	0.92522
112	S112Y	Y	<0.01
112	S112R	R	<0.01
112	S112P	P	<0.01
112	S112H	H	0.380254
112	S112V	V	0.479716
112	S112M	M	0.564157
112	S112W	W	0.582165
112	S112K	K	0.678369
112	S112T	T	0.721644
112	S112N	N	0.850159
112	S112F	F	0.878895
112	S112A	A	0.943049
113	V113S	S	0.572415
113	V113G	G	0.579385
113	V113K	K	0.716865
113	V113H	H	0.763416
113	V113W	W	0.803685
113	V113L	L	0.854963
113	V113T	T	0.861744
113	V113D	D	0.871104
113	V113E	E	0.936465
113	V113C	C	0.937598
113	V113F	F	0.959822
113	V113Y	Y	0.981976
114	L114H	H	<0.01
114	L114E	E	<0.01
114	L114F	F	<0.01
114	L114K	K	<0.01
114	L114R	R	<0.01
114	L114W	W	<0.01
114	L114Y	Y	<0.01
114	L114Q	Q	0.115737
114	L114P	P	0.275464
114	L114S	S	0.545726
114	L114V	V	0.595416
114	L114N	N	0.77333
115	V115H	H	<0.01
115	V115K	K	<0.01
115	V115I	I	0.994833

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
116	T116Y	Y	0.466112
116	T116V	V	0.571817
116	T116R	R	0.619823
116	T116L	L	0.681201
116	T116W	W	0.748358
116	T116I	I	0.760474
116	T116Q	Q	0.768867
116	T116P	P	0.836786
116	T116G	G	0.901886
116	T116E	E	0.906124
116	T116A	A	0.952003
116	T116S	S	0.963005
117	Q117W	W	0.707035
117	Q117V	V	0.761971
117	Q117G	G	0.794858
117	Q117S	S	0.86512
118	V118K	K	<0.01
118	V118W	W	<0.01
118	V118E	E	<0.01
118	V118R	R	0.069623
118	V118P	P	0.222399
118	V118D	D	0.40168
118	V118I	I	0.545694
118	V118G	G	0.559239
118	V118S	S	0.815888
118	V118A	A	0.852723
118	V118T	T	0.91759
118	V118M	M	0.933469
118	V118F	F	0.998467

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
119	L119G	G	<0.01
119	L119S	S	<0.01
119	L119F	F	<0.01
119	L119R	R	<0.01
119	L119P	P	<0.01
119	L119T	T	0.102922
119	L119N	N	0.113151
119	L119V	V	0.150373
119	L119W	W	0.203313
119	L119C	C	0.244106
119	L119D	D	0.280381
119	L119E	E	0.322167
119	L119I	I	0.427476
119	L119H	H	0.462912
119	L119Y	Y	0.556343
120	T120P	P	<0.01
120	T120H	H	0.498304
120	T120R	R	0.599376
120	T120A	A	0.663543
120	T120Q	Q	0.781096
120	T120C	C	0.924433
121	S121P	P	0.384623
121	S121R	R	0.701237
121	S121W	W	0.772781
121	S121K	K	0.77795
121	S121G	G	0.992545
122	A122G	G	<0.01
122	A122D	D	0.059137
122	A122F	F	0.148369

Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
122	A122H	H	0.169443
122	A122R	R	0.396041
122	A122S	S	0.431258
122	A122K	K	0.450105
122	A122E	E	0.467766
122	A122T	T	0.520454
122	A122P	P	0.548155
122	A122I	I	0.647406
122	A122N	N	0.704284
122	A122Q	Q	0.741587
122	A122W	W	0.862265
122	A122V	V	0.886387
122	A122M	M	0.938855
124	G124I	I	<0.01
124	G124H	H	<0.01
124	G124M	M	<0.01
124	G124W	W	<0.01
124	G124P	P	<0.01
124	G124A	A	0.031196
124	G124Q	Q	0.208313
124	G124T	T	0.315233
124	G124V	V	0.329769
124	G124R	R	0.409769
124	G124L	L	0.536625
124	G124S	S	0.555215
124	G124Y	Y	0.559199
124	G124N	N	0.599171
124	G124D	D	0.63784
124	G124C	C	0.672179
124	G124F	F	0.950801
125	V125W	W	0.24527
125	V125E	E	0.385171
125	V125R	R	0.466062
125	V125C	C	0.541228
125	V125D	D	0.541318
125	V125P	P	0.622352
125	V125F	F	0.627367
125	V125S	S	0.790998
125	V125Y	Y	0.813593
125	V125A	A	0.925641
125	V125I	I	0.941326
126	G126I	I	<0.010426347441542
126	G126V	V	0.175001
126	G126Y	Y	0.234673
126	G126L	L	0.540613
126	G126A	A	0.552538
126	G126E	E	0.599533
126	G126P	P	0.673809
126	G126T	T	0.737666
126	G126R	R	0.761417
126	G126N	N	0.846727
126	G126S	S	0.902662
126	G126C	C	0.980807
127	T127L	L	<0.01
127	T127E	E	<0.01
127	T127Q	Q	0.151533
127	T127I	I	0.203586

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
127	T127H	H	0.60105
127	T127D	D	0.61747
127	T127M	M	0.639504
127	T127C	C	0.653314
127	T127V	V	0.683337
127	T127G	G	0.710564
127	T127P	P	0.773291
127	T127S	S	0.828003
128	T128D	D	0.662836
129	Y129W	W	<0.01
129	Y129G	G	<0.01
129	Y129K	K	<0.01
129	Y129V	V	<0.01
129	Y129T	T	0.138769
129	Y129A	A	0.173554
129	Y129R	R	0.178362
129	Y129M	M	0.211662
129	Y129D	D	0.228506
129	Y129L	L	0.270643
129	Y129N	N	0.530034
129	Y129P	P	0.588917
129	Y129C	C	0.610384
129	Y129S	S	0.692051
129	Y129F	F	0.713199
146	P146W	W	0.680806
146	P146T	T	0.756105
146	P146V	V	0.768041
146	P146S	S	0.956673
148	P148Q	Q	0.975963
149	W149R	R	<0.01
149	W149E	E	<0.01
149	W149P	P	<0.01
149	W149C	C	0.1164
149	W149I	I	0.235936
149	W149A	A	0.311848
149	W149S	S	0.329233
149	W149Q	Q	0.402387
149	W149T	T	0.440303
149	W149G	G	0.44856
149	W149M	M	0.494615
149	W149F	F	0.495779
149	W149L	L	0.637667
149	W149Y	Y	0.747652
150	F150P	P	0.31768
150	F150N	N	0.362798
150	F150G	G	0.458431
150	F150V	V	0.511676
150	F150A	A	0.539571
150	F150T	T	0.580879
150	F150W	W	0.622886
150	F150M	M	0.625886
150	F150E	E	0.727755
150	F150C	C	0.778063
150	F150I	I	0.78431
150	F150K	K	0.848249
153	I153N	N	0.890296
154	F154T	T	<0.01
154	F154D	D	<0.01

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Table 10-5. PAD Assay Results

Position	WT/Pos/ Mutation	Variant	PAD Perf. Ind.
154	F154E	E	<0.01
154	F154G	G	<0.01
154	F154L	L	<0.01
154	F154P	P	<0.01
154	F154V	V	<0.01
154	F154S	S	0.287767
154	F154Q	Q	0.973299
194	I194S	S	<0.01
194	I194A	A	<0.01
194	I194C	C	<0.01
194	I194P	P	<0.01
194	I194F	F	<0.01
194	I194W	W	<0.01
194	I194R	R	<0.01
194	I194Y	Y	<0.01
194	I194G	G	0.044503
194	I194L	L	0.577811
194	I194V	V	0.780569
196	F196H	H	<0.01
196	F196G	G	<0.01
196	F196S	S	<0.01
196	F196Q	Q	<0.01
196	F196A	A	<0.01
196	F196K	K	<0.01
196	F196N	N	<0.01
196	F196R	R	<0.01
196	F196W	W	0.38122
196	F196P	P	0.385754
196	F196V	V	0.675769
196	F196M	M	0.709899
196	F196Y	Y	0.970105

The following Table provides variants that are better than wild-type at degrading peracids (*i.e.*, the performance index for the variant is better than the wild-type).

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
1	M001I	1.19
1	M001L	2.11
2	A002D	1.05
2	A002R	1.17
2	A002W	1.17
2	A002P	1.17
2	A002Q	1.29
2	A002E	1.38
3	K003T	1.03
3	K003S	1.17
3	K003Q	1.19
3	K003R	1.29
3	K003Y	1.39
3	K003M	1.44
3	K003P	1.45
3	K003C	1.52
3	K003L	1.84
3	K003H	1.89
3	K003A	2.14
3	K003I	2.44
3	K003E	3.51
3	K003G	3.74
4	R004D	1.18
4	R004C	1.34
4	R004P	1.44
4	R004A	1.64
5	I005M	1.09
5	I005E	1.59
5	I005L	1.63
5	I005A	1.88
5	I005C	2.47
5	I005D	3.11
6	L006C	1.22
6	L006M	1.44
6	L006A	1.99
7	C007A	1.03
7	C007H	1.37
7	C007I	1.48
7	C007E	1.63
7	C007K	2.95
8	F008M	1.11
8	F008L	1.31
8	F008A	1.33
8	F008C	4.01
10	D010L	2.04
13	T013I	1.05
13	T013E	1.09
13	T013L	1.47
13	T013M	1.47
13	T013C	1.55
13	T013A	1.88
13	T013N	2.61

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
13	T013P	2.73
16	W016K	1.03
16	W016I	1.06
16	W016Y	1.09
16	W016L	1.16
17	V017S	1.04
18	P018N	1.42
18	P018Q	3.26
18	P018R	3.97
18	P018C	4.16
18	P018Y	4.17
18	P018V	4.85
18	P018E	4.87
18	P018G	4.96
18	P018H	6.05
18	P018L	7.40
20	E020D	1.14
20	E020S	1.18
20	E020H	1.20
20	E020T	1.25
20	E020V	1.27
20	E020A	1.28
20	E020W	1.30
20	E020N	1.34
20	E020P	1.43
20	E020Q	1.56
20	E020C	1.76
21	D021S	1.11
21	D021E	1.39
21	D021F	1.41
21	D021W	1.44
21	D021L	1.57
21	D021A	1.75
21	D021G	1.76
21	D021K	1.80
21	D021Y	2.01
22	G022I	1.03
22	G022T	1.16
22	G022E	1.19
22	G022L	1.35
22	G022P	1.36
22	G022Q	1.44
22	G022A	1.66
23	A023H	1.04
23	A023L	1.30
24	P024C	1.04
24	P024K	1.36
24	P024L	1.51
26	E026M	1.10
26	E026H	1.19
26	E026D	1.39
26	E026A	1.45
26	E026K	1.47
26	E026L	1.71
27	R027I	1.41
27	R027K	1.55
27	R027L	2.60
27	R027A	2.78
28	F028E	1.04
28	F028W	1.17
28	F028C	1.21
28	F028Y	1.36
28	F028M	1.37
28	F028A	1.48
28	F028L	2.02
28	F028D	2.07
29	A029C	1.15
30	P030H	1.08

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
30	P030G	1.09
30	P030R	1.14
30	P030L	1.17
30	P030E	1.24
30	P030Y	1.31
30	P030I	1.38
30	P030K	1.39
30	P030S	1.49
30	P030T	1.64
30	P030V	1.74
31	D031V	1.08
31	D031T	1.11
31	D031Q	1.13
31	D031W	1.14
31	D031G	1.16
31	D031A	1.18
31	D031S	1.23
31	D031F	1.39
31	D031R	1.49
31	D031N	1.55
31	D031L	1.61
32	V032S	1.09
32	V032N	1.61
32	V032W	1.71
32	V032Q	1.74
32	V032G	2.65
32	V032M	3.41
32	V032I	3.51
32	V032A	3.64
32	V032E	3.92
32	V032D	4.19
32	V032L	4.72
32	V032K	4.73
33	R033S	1.01
33	R033N	1.30
33	R033A	1.32
33	R033C	1.73
33	R033G	2.63
33	R033K	2.72
33	R033L	2.90
34	W034P	1.21
34	W034M	1.22
34	W034C	1.49
34	W034A	2.29
35	T035M	2.72
35	T035A	3.85
35	T035C	4.72
35	T035I	5.38
35	T035E	5.73
36	G036C	1.06
36	G036A	1.07
36	G036H	1.10
36	G036K	1.71
36	G036I	1.81
36	G036L	2.49
36	G036D	2.50
37	V037I	1.04
37	V037L	1.16
37	V037S	1.49
37	V037N	1.52
37	V037C	1.63
37	V037A	2.00
37	V037P	2.10
38	L038V	1.12
39	A039W	1.02
39	A039Y	1.13
40	Q040N	1.00
40	Q040I	1.10

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
40	Q040E	1.28
40	Q040R	1.48
40	Q040L	1.49
40	Q040D	1.59
40	Q040S	1.65
40	Q040T	1.81
40	Q040Y	2.02
40	Q040G	2.17
40	Q040W	2.59
40	Q040K	3.64
41	Q041G	1.09
41	Q041H	1.14
41	Q041R	1.27
41	Q041K	1.61
41	Q041L	1.92
41	Q041A	2.58
42	L042F	1.02
42	L042P	1.34
42	L042K	1.41
42	L042C	1.43
43	G043A	1.07
43	G043L	1.82
43	G043E	1.88
44	A044C	1.92
45	D045F	1.04
46	F046C	1.16
46	F046A	1.25
46	F046E	1.31
46	F046D	1.39
46	F046M	1.42
46	F046K	1.46
46	F046P	1.50
46	F046L	1.54
47	E047L	1.02
47	E047K	1.06
47	E047G	1.10
47	E047I	1.15
48	V048Q	1.39
48	V048F	1.42
48	V048A	1.63
48	V048M	1.79
48	V048C	2.25
48	V048L	2.29
48	V048P	3.08
49	I049Y	1.02
49	I049M	1.02
49	I049L	1.03
49	I049G	1.12
49	I049K	1.26
49	I049A	1.87
50	E050P	1.02
50	E050M	1.04
50	E050G	1.11
50	E050D	1.22
50	E050A	1.23
51	E051T	1.17
51	E051M	1.20
51	E051D	1.28
51	E051G	1.34
51	E051K	2.00
51	E051A	2.72
52	G052W	2.47
53	L053H	1.70
54	S054N	1.29
54	S054P	1.30
54	S054A	1.41
55	A055N	1.05
55	A055K	1.08

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
55	A055C	1.26
57	T057S	1.01
57	T057G	1.05
58	T058L	1.12
58	T058H	1.49
59	N059Q	1.86
59	N059T	5.63
59	N059S	7.32
59	N059K	8.21
59	N059E	9.88
59	N059V	9.97
59	N059G	10.00
59	N059F	10.23
59	N059A	10.44
59	N059Y	11.14
59	N059C	11.23
59	N059D	11.72
59	N059W	12.80
59	N059L	14.74
60	I060G	1.04
60	I060V	1.06
60	I060H	1.07
60	I060Y	1.19
61	D061P	1.13
61	D061Q	1.16
61	D061L	1.20
61	D061G	1.25
61	D061S	1.35
61	D061R	1.59
61	D061I	1.66
61	D061H	1.67
61	D061K	1.72
63	P063K	1.02
63	P063V	1.04

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
63	P063Q	1.05
63	P063W	1.11
63	P063G	1.22
63	P063L	1.23
63	P063T	1.32
64	T064G	1.08
64	T064M	1.09
64	T064A	1.20
64	T064L	1.22
66	P066S	1.02
66	P066T	1.10
69	N069D	1.11
69	N069A	1.13
69	N069Q	1.14
69	N069C	1.20
69	N069L	1.20
69	N069S	1.42
69	N069T	1.43
69	N069H	1.52
69	N069K	1.59
69	N069V	1.73
69	N069I	1.75
70	G070L	1.01
70	G070A	1.41
70	G070H	1.90
71	A071K	1.01
71	A071M	1.11
72	S072F	1.15
72	S072G	1.76
72	S072M	2.13
72	S072C	2.18
72	S072H	2.48
72	S072N	2.85
72	S072A	3.52

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
73	Y073M	1.13
73	Y073C	1.20
73	Y073A	1.40
74	L074F	1.13
74	L074M	1.21
74	L074A	2.90
75	P075E	1.19
75	P075L	1.19
75	P075W	1.31
75	P075Y	1.32
75	P075V	1.39
75	P075C	1.42
75	P075D	2.09
76	S076C	1.06
76	S076T	1.11
76	S076A	1.11
76	S076H	1.11
76	S076P	1.20
76	S076V	1.35
76	S076K	1.53
76	S076M	1.61
76	S076D	1.94
76	S076E	2.09
76	S076G	2.15
76	S076L	4.70
77	C077T	1.03
77	C077D	1.05
78	L078T	1.10
78	L078I	1.11
78	L078G	1.38
78	L078H	1.57
80	T080V	1.01
80	T080Q	1.07
80	T080A	1.11

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
80	T080C	1.15
80	T080S	1.40
80	T080G	1.50
81	H081N	1.00
81	H081L	1.03
81	H081W	1.09
81	H081C	1.09
81	H081A	1.45
81	H081M	1.54
82	L082M	1.06
83	P083C	1.01
83	P083R	1.09
83	P083N	1.10
83	P083K	1.16
83	P083E	1.26
83	P083M	1.88
83	P083A	2.36
84	L084F	1.01
84	L084G	1.01
85	D085R	1.03
85	D085A	1.09
85	D085H	1.24
85	D085E	1.25
85	D085C	1.50
85	D085G	1.60
85	D085F	1.98
86	L086C	2.44
86	L086A	3.32
87	V087P	1.64
87	V087C	2.22
87	V087L	4.30
88	I088M	1.09
88	I088P	3.51
89	I089L	1.22

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
89	I089A	1.83
89	I089P	1.91
90	M090C	1.09
90	M090E	1.15
90	M090A	1.41
90	M090D	2.88
91	L091I	1.05
91	L091C	1.27
91	L091A	1.45
91	L091D	1.47
92	G092C	2.05
93	T093A	1.05
96	T096F	1.24
96	T096G	1.28
96	T096L	1.93
96	T096M	2.53
96	T096C	3.76
96	T096A	4.20
98	A098Y	1.15
98	A098P	1.26
98	A098N	1.40
98	A098C	1.42
98	A098L	1.47
98	A098D	2.19
100	F100C	1.28
100	F100T	1.42
100	F100N	1.45
100	F100A	2.02
100	F100M	2.19
101	R101L	1.12
102	R102Q	1.19
102	R102Y	1.29
102	R102L	1.64
102	R102A	1.79
104	P104V	1.02
104	P104H	1.03
104	P104N	1.44
104	P104C	1.83
104	P104E	1.97
104	P104I	2.05
104	P104M	2.24
105	L105Q	1.04
105	L105H	1.23
105	L105R	1.25
105	L105G	1.40
105	L105W	1.71
105	L105F	1.73
105	L105C	1.92
106	D106S	1.02
106	D106W	1.07
106	D106E	1.09
106	D106C	1.10
106	D106A	1.13
106	D106H	1.18
106	D106K	1.24
106	D106T	1.38
106	D106F	1.45
106	D106G	1.45
106	D106V	1.68
107	I107L	1.04
107	I107S	1.33
107	I107C	1.41
107	I107T	1.53
108	A108S	1.00
108	A108G	1.13
108	A108L	2.56
108	A108K	2.97
110	G110A	1.01

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
110	G110D	1.40
110	G110C	1.43
110	G110E	1.76
110	G110F	2.29
111	M111C	1.01
111	M111A	1.02
111	M111I	1.03
111	M111Y	1.06
111	M111W	1.23
111	M111N	1.31
112	S112L	1.00
112	S112E	1.16
113	V113M	1.06
113	V113Q	1.11
113	V113R	1.11
113	V113P	1.14
113	V113N	1.22
113	V113A	1.31
114	L114T	1.05
114	L114A	1.07
114	L114G	1.14
114	L114C	1.14
114	L114I	1.17
114	L114M	1.28
115	V115C	1.08
115	V115S	1.14
115	V115Q	1.15
115	V115A	1.19
115	V115T	1.28
115	V115L	1.30
115	V115M	1.32
115	V115R	1.63
115	V115F	1.69
115	V115G	1.76
115	V115Y	2.07
115	V115D	2.21
115	V115P	2.21
115	V115W	2.48
116	T116N	1.05
116	T116C	1.05
116	T116H	1.08
116	T116M	1.39
117	Q117F	1.02
117	Q117R	1.05
117	Q117T	1.10
117	Q117H	1.12
117	Q117Y	1.13
117	Q117P	1.13
117	Q117E	1.21
117	Q117A	1.73
117	Q117M	1.89
118	V118L	1.05
118	V118C	1.14
118	V118Y	1.34
118	V118Q	1.50
119	L119A	1.02
120	T120V	1.07
120	T120S	1.07
120	T120K	1.09
120	T120M	1.22
120	T120L	1.26
120	T120N	1.42
120	T120E	1.53
120	T120I	1.56
120	T120Y	1.61
121	S121E	1.04
121	S121N	1.06
121	S121Q	1.09

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
121	S121T	1.26
121	S121L	1.49
121	S121A	1.55
121	S121V	1.59
121	S121C	1.64
122	A122L	1.02
123	G123K	1.12
123	G123A	1.19
123	G123Y	1.24
123	G123M	1.38
123	G123L	1.38
123	G123W	1.39
125	V125G	1.09
126	G126M	1.17
126	G126D	1.22
127	T127A	1.10
128	T128M	1.06
128	T128H	1.08
128	T128V	1.15
128	T128P	1.16
128	T128W	1.23
128	T128S	1.27
128	T128A	1.31
128	T128Q	1.34
128	T128N	1.36
128	T128K	1.57
128	T128R	1.70
128	T128F	1.71
128	T128L	1.72
128	T128Y	1.81
131	A131R	1.04
132	P132N	1.05
132	P132L	2.24
132	P132E	3.02
132	P132Y	4.78
132	P132G	4.98
132	P132S	5.05
132	P132C	5.68
132	P132A	6.08
132	P132Q	6.15
133	K133Y	1.44
133	K133L	1.92
134	V134C	1.37
134	V134G	1.42
134	V134S	1.44
134	V134L	1.45
134	V134A	1.64
134	V134P	1.71
134	V134M	1.89
134	V134N	2.80
135	L135D	2.90
136	V136T	1.13
136	V136L	1.13
136	V136C	1.23
136	V136A	1.60
137	V137M	1.13
137	V137L	1.27
137	V137C	1.42
137	V137A	1.46
138	S138G	1.11
138	S138C	1.18
138	S138A	1.28
138	S138N	1.31
138	S138P	1.39
140	P140C	1.07
140	P140A	1.83
140	P140H	2.25
140	P140F	2.89

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
140	P140G	3.11
141	P141A	1.08
143	A143C	1.07
143	A143E	1.13
143	A143D	1.22
143	A143L	1.28
143	A143H	1.36
143	A143K	1.37
144	P144M	1.01
144	P144F	1.08
144	P144Q	1.08
144	P144K	1.09
144	P144R	1.14
144	P144L	1.15
144	P144D	1.38
144	P144N	1.49
144	P144H	1.60
144	P144Y	1.65
146	P146N	1.00
146	P146G	1.04
146	P146R	1.06
146	P146M	1.23
146	P146A	1.36
146	P146Y	1.44
146	P146F	1.53
146	P146H	1.57
146	P146C	1.69
146	P146L	2.00
147	H147Q	1.03
147	H147W	1.05
147	H147K	1.06
147	H147E	1.10
147	H147Y	1.12
147	H147C	1.17

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
147	H147D	1.18
147	H147P	1.21
147	H147N	1.25
147	H147L	1.29
147	H147M	1.44
148	P148V	1.04
148	P148A	1.06
148	P148T	1.09
148	P148E	1.19
148	P148G	1.20
148	P148S	1.21
148	P148R	1.25
148	P148K	1.30
148	P148D	1.34
148	P148Y	1.37
148	P148L	1.39
148	P148F	1.50
149	W149H	1.01
150	F150Y	1.07
150	F150H	1.18
150	F150L	1.30
151	Q151P	1.91
151	Q151E	2.07
151	Q151K	2.19
151	Q151H	2.19
151	Q151S	2.25
151	Q151R	2.32
151	Q151T	2.37
151	Q151C	2.55
151	Q151Y	2.75
151	Q151D	2.81
151	Q151A	2.93
151	Q151M	6.36
152	L152M	1.10

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Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
152	L152C	1.14
152	L152E	1.23
152	L152A	1.29
152	L152Y	1.37
152	L152W	1.55
153	I153V	1.15
153	I153A	1.49
153	I153L	1.50
153	I153T	1.62
153	I153S	1.66
153	I153F	1.75
153	I153P	1.87
153	I153H	2.00
153	I153K	2.44
154	F154Y	4.96
155	E155S	1.12
155	E155G	1.12
155	E155T	1.19
155	E155D	1.24
155	E155K	1.33
155	E155N	1.79
155	E155L	2.07
155	E155A	2.59
155	E155P	2.60
155	E155Y	2.65
155	E155M	2.91
156	G156S	1.04
156	G156K	1.11
156	G156E	1.14
156	G156R	1.21
156	G156A	1.21
156	G156P	1.29
156	G156C	1.37
156	G156N	1.38
156	G156H	1.40
156	G156Y	1.40
156	G156T	1.53
156	G156M	1.62
156	G156D	1.62
157	G157I	1.33
157	G157F	1.42
157	G157K	1.47
157	G157H	1.57
158	E158H	1.01
158	E158P	1.19
158	E158Q	1.24
158	E158S	1.27
158	E158A	1.28
158	E158R	1.29
158	E158W	1.31
158	E158C	1.37
158	E158N	1.58
158	E158M	1.73
158	E158F	1.77
158	E158K	1.88
158	E158L	1.96
158	E158Y	2.48
159	Q159H	1.48
160	K160N	1.12
160	K160A	1.14
160	K160R	1.15
160	K160D	1.19
160	K160C	1.29
160	K160Q	1.41
160	K160M	1.47
160	K160P	1.66
161	T161L	1.16
161	T161V	1.24

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
161	T161Q	1.50
161	T161M	1.72
161	T161Y	2.62
162	T162R	1.23
162	T162G	1.82
162	T162S	2.01
162	T162W	2.04
162	T162I	2.21
162	T162Q	2.45
162	T162Y	2.89
162	T162K	3.13
162	T162F	3.23
162	T162M	3.49
162	T162C	3.57
162	T162L	3.59
162	T162N	3.84
162	T162H	3.91
162	T162P	4.37
163	E163N	1.00
163	E163C	1.08
163	E163D	1.08
163	E163A	1.79
163	E163Y	1.89
163	E163L	1.94
164	L164Q	1.01
164	L164V	1.02
164	L164S	1.11
164	L164M	1.26
164	L164N	1.31
164	L164R	1.61
164	L164P	2.41
165	A165G	1.07
165	A165V	1.13
165	A165N	1.20
165	A165R	1.29
165	A165Q	1.32
165	A165T	1.32
165	A165P	1.34
165	A165C	1.42
165	A165L	1.55
165	A165M	1.56
165	A165D	1.69
166	R166W	1.08
166	R166F	1.10
166	R166K	1.20
166	R166N	1.21
166	R166Y	1.22
166	R166M	1.29
166	R166I	1.39
166	R166P	1.50
166	R166L	1.50
166	R166A	1.51
166	R166D	1.55
166	R166H	1.56
167	V167I	1.00
167	V167S	1.86
167	V167H	2.11
167	V167Y	2.15
167	V167R	2.25
167	V167Q	2.41
167	V167T	2.47
167	V167L	2.56
167	V167G	2.83
167	V167M	3.84
167	V167A	4.99
167	V167C	5.37
167	V167D	5.54
167	V167P	6.08

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
168	Y168F	5.17
168	Y168L	5.39
169	S169Y	1.10
169	S169A	1.13
169	S169R	1.19
169	S169K	1.27
169	S169Q	1.37
169	S169C	1.38
169	S169M	1.40
169	S169L	1.47
169	S169I	1.53
170	A170C	1.06
170	A170E	1.17
170	A170F	1.17
170	A170N	1.17
170	A170M	1.28
170	A170D	1.32
170	A170P	1.33
171	L171H	1.07
171	L171G	1.33
171	L171Y	1.35
171	L171T	1.36
171	L171V	1.39
171	L171I	1.42
171	L171K	1.53
171	L171A	1.66
171	L171C	1.73
171	L171S	1.76
171	L171Q	1.93
171	L171F	1.97
171	L171M	2.22
171	L171N	2.79
172	A172M	1.06
172	A172L	1.22

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
172	A172D	1.42
172	A172Y	1.76
173	S173T	1.29
173	S173H	1.49
173	S173I	2.22
173	S173F	2.30
173	S173R	2.47
173	S173V	2.54
173	S173E	2.65
173	S173P	2.66
173	S173A	2.72
173	S173M	3.01
173	S173K	3.01
173	S173C	3.07
173	S173Y	3.54
173	S173W	3.67
173	S173L	3.86
174	F174H	1.05
174	F174K	1.17
174	F174P	1.46
174	F174Y	1.66
174	F174L	1.83
174	F174A	2.09
174	F174M	2.20
175	M175N	1.02
175	M175E	1.43
176	K176C	1.01
176	K176R	1.03
176	K176E	1.08
176	K176W	1.16
176	K176D	1.18
176	K176A	1.19
176	K176F	1.28
176	K176V	1.33

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
176	K176M	1.33
178	P178K	1.70
178	P178T	2.28
178	P178V	2.70
178	P178G	2.95
178	P178S	3.06
178	P178Q	3.64
178	P178M	3.87
178	P178E	4.15
178	P178A	4.39
178	P178D	6.44
178	P178Y	6.91
178	P178L	7.15
179	F179G	1.16
179	F179V	1.17
179	F179Y	1.47
179	F179E	1.80
179	F179L	1.89
180	F180W	1.81
180	F180C	1.94
180	F180I	2.11
180	F180L	2.13
180	F180A	2.70
180	F180Y	2.99
180	F180N	3.05
180	F180V	3.24
180	F180M	4.36
181	D181A	1.23
183	G183P	1.02
183	G183R	1.09
183	G183Y	1.45
183	G183L	1.50
183	G183C	1.99
184	S184Y	1.09

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
184	S184Q	1.16
184	S184I	1.21
184	S184V	1.25
184	S184F	1.27
184	S184K	1.61
184	S184A	1.69
184	S184M	1.77
184	S184E	1.86
184	S184N	1.93
184	S184L	2.00
184	S184D	2.24
184	S184C	2.39
185	V185F	1.20
185	V185Q	1.41
185	V185M	1.46
186	I186L	1.14
186	I186M	1.38
186	I186A	1.79
186	I186D	4.29
187	S187K	1.16
187	S187D	1.40
187	S187G	1.46
187	S187L	1.46
187	S187H	1.51
187	S187I	1.58
187	S187N	1.59
187	S187C	1.67
187	S187A	1.72
187	S187M	1.87
188	T188N	1.69
188	T188E	1.97
189	D189A	1.18
189	D189T	1.21
189	D189I	1.27

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
189	D189L	1.30
190	G190C	1.17
190	G190Y	1.39
190	G190P	1.86
190	G190D	2.02
190	G190H	2.92
190	G190A	3.42
190	G190M	5.54
191	V191T	1.03
191	V191R	1.91
191	V191K	2.17
191	V191F	2.75
191	V191C	2.81
191	V191Y	4.34
191	V191L	4.69
191	V191A	5.06
191	V191E	5.46
191	V191Q	5.83
191	V191D	6.03
191	V191M	7.34
193	G193S	1.60
193	G193E	3.15
193	G193Q	4.29
193	G193V	5.21
195	H195P	1.16
195	H195M	1.28
195	H195K	1.33
195	H195Y	1.49
195	H195E	1.70
195	H195D	1.93
196	F196I	1.12
196	F196L	1.17
196	F196C	1.18
197	T197H	1.24

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
197	T197A	1.42
197	T197M	2.38
198	E198T	1.16
198	E198S	1.18
198	E198F	1.21
198	E198V	1.44
198	E198Q	1.46
198	E198A	1.46
198	E198I	1.48
198	E198L	1.54
198	E198N	1.67
198	E198P	1.72
198	E198Y	1.77
198	E198W	1.78
198	E198C	1.83
198	E198M	1.86
198	E198R	1.88
199	A199F	1.15
199	A199H	1.15
199	A199R	1.17
199	A199T	1.22
199	A199E	1.31
199	A199D	1.33
199	A199V	1.45
199	A199K	1.53
199	A199Y	1.59
199	A199L	1.65
199	A199C	2.45
201	N201D	1.64
202	R202M	1.76
202	R202G	1.82
202	R202S	1.84
202	R202C	1.93
202	R202A	1.97

Table 10-6. Variants with Peracid Degradation Greater Than Wild-Type

Pos.	WT/Pos./Var.	PAD PI
202	R202I	1.99
202	R202E	2.05
202	R202L	2.05
202	R202T	2.06
202	R202H	2.09
202	R202F	2.16
202	R202W	2.52
203	D203Q	1.03
203	D203S	1.13
203	D203I	1.19
203	D203N	1.28
203	D203G	1.33
203	D203F	1.34
203	D203H	1.54
203	D203P	1.71
203	D203R	1.77
203	D203A	1.96
203	D203L	2.08
203	D203C	2.09

The following Table provides variants that exhibited peracid degradation that was less than wild-type.

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
1	M001V	0.94
2	A002Y	0.46
2	A002N	0.59
2	A002V	0.60
2	A002I	0.61
2	A002T	0.61
2	A002S	0.66
2	A002G	0.84
2	A002F	0.93
3	K003V	0.84
4	R004L	0.01
4	R004V	0.08

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
4	R004I	0.15
4	R004W	0.48
4	R004G	0.79
4	R004S	0.91
4	R004E	0.97
4	R004Y	0.98
4	R004H	0.99
4	R004Q	0.99
4	R004T	1.00
5	I005G	0.01
5	I005N	0.01
5	I005P	0.01
5	I005R	0.01
5	I005F	0.15
5	I005S	0.37
5	I005H	0.63
5	I005T	0.72
5	I005V	0.92
6	L006S	0.01
6	L006K	0.01
6	L006G	0.01
6	L006H	0.01
6	L006R	0.01
6	L006W	0.01
6	L006E	0.01
6	L006Q	0.01
6	L006V	0.35
6	L006T	0.35
6	L006I	0.82
7	C007S	0.01
7	C007R	0.01
7	C007Y	0.54
7	C007M	0.68
7	C007G	0.69

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
8	F008S	0.01
8	F008R	0.46
8	F008H	0.64
8	F008G	0.65
8	F008T	0.77
8	F008K	0.83
8	F008P	0.83
8	F008V	0.85
8	F008Y	0.90
8	F008N	0.96
9	G009H	0.01
9	G009T	0.01
10	D010W	0.01
10	D010K	0.01
10	D010Y	0.01
10	D010T	0.01
10	D010I	0.01
10	D010V	0.01
10	D010S	0.01
10	D010G	0.01
10	D010R	0.01
10	D010A	0.01
10	D010M	0.01
10	D010N	0.01
10	D010P	0.01
10	D010E	0.15
11	S011T	0.01
11	S011V	0.01
11	S011D	0.01
11	S011E	0.01
11	S011F	0.01
11	S011G	0.01
11	S011L	0.01
11	S011Q	0.01

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
11	S011R	0.01
11	S011H	0.33
11	S011K	0.40
11	S011A	0.53
11	S011I	0.56
12	L012V	0.01
12	L012S	0.01
12	L012G	0.01
12	L012R	0.01
12	L012D	0.01
12	L012P	0.01
12	L012W	0.02
12	L012T	0.06
12	L012A	0.07
12	L012K	0.13
12	L012H	0.16
12	L012F	0.17
12	L012Q	0.22
12	L012C	0.22
12	L012N	0.66
13	T013Q	0.51
13	T013V	0.63
13	T013S	0.68
13	T013G	0.77
14	W014I	0.01
14	W014S	0.01
14	W014G	0.01
14	W014K	0.01
14	W014V	0.01
14	W014L	0.01
14	W014T	0.01
14	W014R	0.01
14	W014N	0.01
14	W014P	0.01
14	W014E	0.15
14	W014F	0.22
14	W014A	0.27
14	W014Y	0.66
15	G015C	0.01
15	G015N	0.01
15	G015D	0.01
15	G015E	0.01
15	G015P	0.01
15	G015A	0.61
15	G015S	0.63
16	W016S	0.01
16	W016G	0.01
16	W016H	0.01
16	W016T	0.01
16	W016R	0.01
16	W016N	0.01
16	W016P	0.15
16	W016Q	0.31
16	W016M	0.37
16	W016A	0.55
16	W016D	0.57
16	W016E	0.65
16	W016V	0.88
17	V017A	0.68
17	V017E	0.75
17	V017G	0.84
17	V017K	0.84
17	V017F	0.85
17	V017T	0.86
17	V017Y	0.88
17	V017R	0.94
17	V017P	0.96
17	V017I	0.99

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
17	V017L	1.00
18	P018S	0.07
19	V019P	0.01
19	V019M	0.12
19	V019R	0.34
19	V019Q	0.40
19	V019A	0.55
19	V019G	0.56
19	V019S	0.57
19	V019E	0.62
19	V019Y	0.70
19	V019D	0.79
19	V019L	0.91
19	V019K	0.97
20	E020L	0.73
20	E020G	0.78
21	D021P	0.86
22	G022K	0.01
22	G022W	0.23
22	G022R	0.56
22	G022V	0.85
22	G022S	0.98
23	A023R	0.28
23	A023S	0.34
23	A023G	0.35
23	A023F	0.44
23	A023V	0.60
23	A023Q	0.73
23	A023P	0.73
23	A023W	0.80
23	A023M	0.95
23	A023Y	0.96
24	P024S	0.61
24	P024Q	0.65
24	P024T	0.66
24	P024A	0.68
24	P024G	0.76
24	P024I	0.85
24	P024R	0.91
24	P024H	0.97
25	T025P	0.01
25	T025H	0.01
25	T025L	0.01
25	T025R	0.01
25	T025M	0.01
25	T025E	0.01
25	T025D	0.01
25	T025K	0.13
25	T025W	0.14
25	T025I	0.35
25	T025G	0.43
25	T025C	0.51
25	T025V	0.51
25	T025S	0.58
25	T025A	0.86
26	E026S	0.28
26	E026T	0.40
26	E026W	0.47
26	E026N	0.48
26	E026R	0.81
26	E026G	0.87
26	E026C	0.94
26	E026V	0.97
26	E026P	0.99
27	R027W	0.01
27	R027T	0.01
27	R027P	0.48
27	R027C	0.58

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
27	R027S	0.69
27	R027G	0.84
27	R027E	0.93
27	R027V	0.94
28	F028G	0.01
28	F028P	0.39
28	F028V	0.53
28	F028S	0.70
29	A029V	0.44
29	A029T	0.47
29	A029S	0.55
29	A029Y	0.59
29	A029P	0.62
29	A029R	0.73
29	A029W	0.74
29	A029M	0.77
29	A029G	0.80
29	A029E	0.84
29	A029D	1.00
30	P030M	0.79
30	P030Q	0.91
30	P030A	0.92
31	D031E	0.88
32	V032P	0.01
32	V032R	0.72
33	R033V	0.94
34	W034R	0.01
34	W034E	0.01
34	W034Q	0.04
34	W034S	0.08
34	W034T	0.15
34	W034V	0.73
34	W034G	0.88
34	W034I	0.94
35	T035Q	0.01
35	T035N	0.01
35	T035R	0.01
35	T035V	0.34
36	G036S	0.26
36	G036T	0.33
36	G036V	0.38
36	G036M	0.54
36	G036N	0.56
36	G036W	0.68
36	G036Q	0.71
36	G036R	0.90
37	V037T	0.81
37	V037H	0.96
37	V037W	0.98
38	L038K	0.01
38	L038G	0.01
38	L038E	0.01
38	L038P	0.01
38	L038Q	0.01
38	L038R	0.01
38	L038D	0.12
38	L038S	0.29
38	L038A	0.63
38	L038C	0.72
39	A039S	0.01
39	A039G	0.30
39	A039N	0.43
39	A039R	0.64
39	A039I	0.71
39	A039P	0.74
39	A039T	0.79
39	A039M	0.81
39	A039E	0.83

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
39	A039C	0.92
39	A039K	0.96
39	A039L	0.97
39	A039V	0.98
40	Q040P	0.01
41	Q041V	0.01
41	Q041S	0.22
41	Q041P	0.66
41	Q041Y	0.70
41	Q041W	0.88
42	L042W	0.01
42	L042H	0.01
42	L042T	0.01
42	L042Q	0.28
42	L042S	0.45
42	L042R	0.64
42	L042I	0.66
42	L042V	0.73
42	L042M	0.74
42	L042G	0.76
43	G043S	0.23
43	G043P	0.31
43	G043V	0.33
43	G043Q	0.48
43	G043R	0.59
43	G043C	0.73
43	G043I	0.77
43	G043K	0.86
43	G043M	0.88
43	G043Y	0.94
43	G043H	0.96
44	A044S	0.01
44	A044Y	0.01
44	A044T	0.01
44	A044R	0.01
44	A044E	0.03
44	A044V	0.50
44	A044F	0.80
44	A044W	0.85
44	A044M	0.98
44	A044L	0.99
45	D045S	0.38
45	D045T	0.44
45	D045R	0.49
45	D045V	0.50
45	D045P	0.53
45	D045Q	0.57
45	D045W	0.58
45	D045H	0.78
45	D045L	0.78
45	D045M	0.78
45	D045G	0.84
45	D045A	0.84
45	D045C	0.84
45	D045K	0.87
46	F046T	0.43
46	F046W	0.63
46	F046S	0.66
46	F046V	0.79
46	F046I	0.88
46	F046G	0.94
47	E047P	0.36
47	E047R	0.62
47	E047N	0.63
47	E047S	0.63
47	E047M	0.70
47	E047A	0.76
47	E047F	0.76

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
47	E047C	0.77
47	E047T	0.84
47	E047D	0.98
47	E047H	0.99
48	V048R	0.01
48	V048S	0.42
48	V048G	0.87
48	V048N	0.98
48	V048E	0.99
49	I049P	0.16
49	I049R	0.29
49	I049W	0.68
49	I049H	0.74
49	I049S	0.79
49	I049E	0.88
49	I049V	0.97
50	E050R	0.01
50	E050W	0.14
50	E050V	0.43
50	E050I	0.58
50	E050S	0.65
50	E050Q	0.91
50	E050L	0.97
51	E051R	0.01
51	E051I	0.04
51	E051W	0.17
51	E051V	0.37
51	E051Q	0.76
51	E051L	0.93
52	G052H	0.01
52	G052S	0.01
52	G052V	0.01
52	G052T	0.01
52	G052M	0.01
52	G052F	0.01
52	G052I	0.07
52	G052P	0.24
52	G052L	0.24
52	G052Q	0.28
52	G052R	0.35
52	G052E	0.55
52	G052A	0.79
53	L053R	0.01
53	L053W	0.01
53	L053P	0.01
53	L053D	0.01
53	L053E	0.19
53	L053K	0.24
53	L053S	0.26
53	L053G	0.33
53	L053V	0.65
53	L053I	0.66
53	L053Q	0.72
53	L053T	0.84
54	S054F	0.01
54	S054W	0.01
54	S054H	0.01
54	S054K	0.08
54	S054I	0.12
54	S054Y	0.12
54	S054G	0.17
54	S054L	0.26
54	S054V	0.29
54	S054E	0.30
54	S054T	0.33
54	S054R	0.35
54	S054M	0.48
54	S054Q	0.53

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
54	S054D	0.65
54	S054C	0.88
55	A055V	0.01
55	A055I	0.01
55	A055P	0.01
55	A055W	0.01
55	A055Y	0.18
55	A055R	0.25
55	A055T	0.42
55	A055G	0.73
55	A055L	0.87
55	A055S	0.87
55	A055H	0.92
56	R056C	0.01
56	R056G	0.01
56	R056T	0.01
56	R056E	0.01
56	R056Q	0.01
56	R056S	0.12
56	R056L	0.24
56	R056N	0.27
56	R056A	0.69
57	T057R	0.01
57	T057P	0.01
57	T057N	0.25
57	T057C	0.40
57	T057Y	0.55
57	T057H	0.61
57	T057A	0.65
57	T057L	0.76
57	T057V	0.87
57	T057I	0.87
58	T058M	0.03
58	T058A	0.36
58	T058V	0.96
58	T058S	0.96
59	N059R	0.01
59	N059M	0.01
59	N059P	0.01
60	I060P	0.32
60	I060D	0.66
60	I060C	0.67
60	I060M	0.68
60	I060A	0.79
60	I060R	0.81
60	I060L	0.91
60	I060E	0.92
60	I060K	0.96
60	I060S	1.00
61	D061F	0.70
61	D061A	0.71
61	D061C	0.85
61	D061Y	0.95
61	D061V	0.97
61	D061N	1.00
62	D062T	0.01
62	D062I	0.01
62	D062V	0.01
62	D062H	0.01
62	D062W	0.01
62	D062S	0.01
62	D062L	0.01
62	D062G	0.01
62	D062R	0.01
62	D062M	0.01
62	D062P	0.01
62	D062Q	0.01
62	D062A	0.11

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
62	D062C	0.49
62	D062E	0.60
63	P063A	0.60
63	P063R	0.80
63	P063S	0.90
63	P063M	0.91
63	P063F	0.93
63	P063Y	0.95
64	T064R	0.11
64	T064D	0.64
64	T064W	0.69
64	T064Q	0.87
64	T064C	0.88
64	T064P	0.94
64	T064H	0.96
64	T064N	0.98
64	T064S	0.99
65	D065V	0.20
65	D065R	0.22
65	D065H	0.40
65	D065Y	0.42
65	D065P	0.42
65	D065S	0.47
65	D065W	0.50
65	D065T	0.50
65	D065G	0.52
65	D065I	0.62
65	D065A	0.72
66	P066N	0.38
66	P066Q	0.42
66	P066G	0.44
66	P066R	0.51
66	P066C	0.52
66	P066A	0.56
66	P066F	0.67
66	P066Y	0.70
66	P066D	0.72
66	P066I	0.84
66	P066V	0.89
66	P066H	0.95
66	P066L	0.99
67	R067F	0.01
67	R067W	0.02
67	R067P	0.04
67	R067E	0.11
67	R067V	0.12
67	R067Q	0.13
67	R067L	0.16
67	R067A	0.22
67	R067T	0.32
67	R067N	0.33
67	R067G	0.41
67	R067K	0.99
68	L068G	0.01
68	L068A	0.01
68	L068M	0.03
68	L068C	0.06
68	L068S	0.07
68	L068N	0.10
68	L068E	0.13
68	L068H	0.22
68	L068Q	0.25
68	L068F	0.25
68	L068T	0.32
68	L068P	0.35
68	L068D	0.44
68	L068Y	0.45
68	L068R	0.47

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
68	L068V	0.51
68	L068W	0.56
68	L068I	0.73
69	N069Y	0.17
69	N069W	0.55
69	N069P	0.59
69	N069R	0.83
69	N069G	0.98
70	G070M	0.01
70	G070T	0.01
70	G070P	0.01
70	G070V	0.01
70	G070C	0.01
70	G070R	0.01
70	G070Y	0.01
70	G070K	0.01
70	G070N	0.01
70	G070Q	0.01
70	G070F	0.01
70	G070I	0.27
70	G070E	0.33
70	G070S	0.64
71	A071P	0.01
71	A071N	0.61
71	A071D	0.65
71	A071G	0.68
71	A071S	0.69
71	A071R	0.77
71	A071H	0.78
71	A071I	0.79
71	A071T	0.79
71	A071E	0.81
71	A071L	0.84
71	A071F	0.99

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
71	A071C	0.99
72	S072Y	0.07
72	S072W	0.34
72	S072P	0.56
72	S072Q	0.66
72	S072L	0.70
72	S072R	0.74
72	S072D	0.80
72	S072V	0.83
72	S072E	0.93
72	S072T	0.97
73	Y073P	0.01
73	Y073R	0.26
73	Y073L	0.50
73	Y073G	0.51
73	Y073H	0.52
73	Y073I	0.64
73	Y073S	0.68
73	Y073V	0.74
73	Y073N	0.76
73	Y073D	0.80
73	Y073Q	0.87
73	Y073K	0.94
74	L074S	0.01
74	L074G	0.57
74	L074V	0.61
74	L074I	0.64
74	L074W	0.67
74	L074Y	0.86
75	P075M	0.30
75	P075R	0.46
75	P075Q	0.61
75	P075S	0.63
75	P075T	0.69

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
75	P075I	0.74
75	P075H	0.86
75	P075K	0.88
75	P075G	0.93
76	S076W	0.01
76	S076Y	0.18
76	S076F	0.46
76	S076Q	0.90
77	C077Y	0.01
77	C077R	0.01
77	C077W	0.01
77	C077F	0.01
77	C077G	0.18
77	C077L	0.73
77	C077S	0.76
77	C077V	0.80
77	C077A	0.91
78	L078E	0.01
78	L078N	0.01
78	L078M	0.48
78	L078Q	0.52
78	L078C	0.78
78	L078Y	0.81
78	L078V	0.83
79	A079H	0.01
79	A079F	0.01
79	A079C	0.03
79	A079Q	0.27
79	A079E	0.27
79	A079N	0.28
79	A079M	0.28
79	A079R	0.32
79	A079W	0.53
79	A079T	0.60

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
79	A079I	0.67
79	A079S	0.78
79	A079G	0.92
79	A079P	0.94
79	A079L	0.96
80	T080W	0.01
80	T080L	0.01
80	T080K	0.01
80	T080R	0.01
80	T080E	0.01
80	T080P	0.01
80	T080H	0.05
80	T080Y	0.11
80	T080I	0.15
80	T080N	0.53
81	H081R	0.01
81	H081Y	0.14
81	H081K	0.56
81	H081S	0.69
81	H081V	0.71
81	H081P	0.72
81	H081Q	0.75
81	H081G	0.80
81	H081F	0.90
82	L082R	0.01
82	L082S	0.01
82	L082W	0.01
82	L082V	0.19
82	L082G	0.31
82	L082T	0.38
82	L082H	0.47
82	L082I	0.51
82	L082K	0.51
82	L082P	0.52

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
82	L082A	0.98
83	P083T	0.01
83	P083V	0.19
83	P083L	0.21
83	P083H	0.61
83	P083W	0.62
83	P083G	0.68
83	P083S	0.79
83	P083Q	0.82
83	P083D	0.83
83	P083F	0.99
84	L084W	0.01
84	L084V	0.42
84	L084P	0.43
84	L084T	0.44
84	L084A	0.45
84	L084Q	0.52
84	L084S	0.55
84	L084R	0.57
84	L084N	0.67
84	L084K	0.79
84	L084D	0.85
84	L084I	0.87
84	L084H	0.99
85	D085I	0.10
85	D085L	0.24
85	D085V	0.25
85	D085W	0.34
85	D085P	0.54
85	D085Y	0.55
85	D085S	0.68
85	D085T	0.71
85	D085N	0.78
85	D085Q	0.99

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
86	L086H	0.01
86	L086S	0.01
86	L086R	0.01
86	L086E	0.01
86	L086Q	0.01
86	L086W	0.08
86	L086V	0.12
86	L086T	0.28
86	L086G	0.70
86	L086Y	0.82
86	L086P	0.99
87	V087S	0.01
87	V087G	0.01
87	V087Y	0.01
87	V087R	0.01
87	V087K	0.01
87	V087D	0.01
87	V087F	0.10
87	V087T	0.15
87	V087A	0.17
87	V087M	0.75
88	I088H	0.01
88	I088T	0.01
88	I088G	0.01
88	I088N	0.01
88	I088Q	0.01
89	I089H	0.01
89	I089S	0.01
89	I089G	0.01
89	I089W	0.01
89	I089Q	0.01
89	I089E	0.01
89	I089F	0.75
89	I089V	0.82

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
89	I089T	0.90
90	M090S	0.01
90	M090W	0.01
90	M090G	0.01
90	M090P	0.01
90	M090V	0.08
90	M090T	0.15
90	M090R	0.36
90	M090I	0.66
90	M090Q	0.77
90	M090L	0.98
91	L091G	0.01
91	L091T	0.01
91	L091Q	0.01
91	L091E	0.01
91	L091S	0.43
91	L091V	0.79
91	L091M	0.88
92	G092V	0.01
92	G092S	0.01
92	G092E	0.01
92	G092F	0.01
93	T093Q	0.01
93	T093Y	0.03
93	T093D	0.23
93	T093S	0.49
93	T093F	0.54
93	T093C	0.95
94	N094L	0.01
94	N094T	0.01
94	N094V	0.01
94	N094H	0.01
94	N094R	0.01
94	N094W	0.01

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
94	N094M	0.03
94	N094C	0.07
94	N094Y	0.12
94	N094G	0.53
94	N094A	0.74
94	N094P	0.79
94	N094S	0.88
95	D095E	0.75
96	T096I	0.01
96	T096W	0.01
96	T096Y	0.01
96	T096R	0.14
96	T096V	0.59
96	T096S	0.79
96	T096P	0.89
97	K097Q	0.01
97	K097G	0.01
97	K097I	0.01
97	K097W	0.01
97	K097L	0.01
97	K097V	0.01
97	K097Y	0.01
97	K097S	0.01
97	K097T	0.01
97	K097M	0.22
97	K097A	0.23
97	K097P	0.27
97	K097R	0.59
98	A098T	0.27
98	A098G	0.56
98	A098S	0.65
98	A098I	0.65
98	A098H	0.92
99	Y099R	0.29

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
99	Y099V	0.31
99	Y099S	0.37
99	Y099W	0.57
99	Y099H	0.59
99	Y099I	0.61
99	Y099G	0.70
99	Y099P	0.81
99	Y099A	0.82
99	Y099L	0.86
100	F100W	0.01
100	F100K	0.01
100	F100D	0.01
100	F100E	0.15
100	F100S	0.85
101	R101W	0.01
101	R101K	0.07
101	R101Q	0.11
101	R101V	0.44
101	R101D	0.80
101	R101Y	0.80
101	R101P	0.86
101	R101N	0.92
101	R101C	0.95
101	R101I	0.96
101	R101F	0.97
102	R102W	0.01
102	R102F	0.23
102	R102G	0.27
102	R102C	0.36
102	R102V	0.61
102	R102D	0.68
102	R102P	0.89
102	R102S	0.96
103	T103W	0.01
103	T103Y	0.01
103	T103G	0.01
103	T103K	0.01
103	T103I	0.01
103	T103L	0.01
103	T103H	0.01
103	T103A	0.01
103	T103V	0.01
103	T103S	0.01
103	T103C	0.01
103	T103R	0.01
103	T103N	0.01
103	T103F	0.01
103	T103P	0.01
104	P104R	0.01
104	P104W	0.23
104	P104T	0.33
104	P104S	0.53
104	P104Q	0.85
104	P104F	0.86
104	P104G	0.98
105	L105V	0.01
105	L105E	0.53
105	L105S	0.61
105	L105Y	0.62
105	L105T	0.64
105	L105P	0.90
106	D106R	0.56
106	D106Q	0.62
106	D106P	0.63
106	D106N	0.64
106	D106M	0.86
106	D106I	0.92
106	D106L	1.00

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
107	I107E	0.01
107	I107G	0.01
107	I107F	0.01
107	I107Q	0.01
107	I107R	0.01
107	I107P	0.32
107	I107Y	0.52
107	I107A	0.80
107	I107N	0.93
107	I107V	0.97
108	A108E	0.61
108	A108Q	0.73
108	A108T	0.87
108	A108V	0.95
109	L109W	0.01
109	L109D	0.11
109	L109I	0.14
109	L109E	0.19
109	L109R	0.21
109	L109H	0.22
109	L109Q	0.22
109	L109F	0.32
109	L109A	0.32
109	L109S	0.38
109	L109P	0.43
109	L109G	0.51
109	L109V	0.54
109	L109M	0.63
109	L109N	0.66
109	L109T	0.79
109	L109Y	0.83
110	G110T	0.01
110	G110W	0.01
110	G110Y	0.01
110	G110P	0.22
110	G110I	0.23
110	G110S	0.30
110	G110Q	0.34
110	G110R	0.48
110	G110H	0.73
110	G110N	0.77
110	G110M	0.82
111	M111R	0.01
111	M111S	0.14
111	M111H	0.19
111	M111G	0.32
111	M111P	0.57
111	M111E	0.67
111	M111L	0.67
111	M111K	0.71
111	M111T	0.76
111	M111F	0.78
111	M111D	0.79
111	M111V	0.93
112	S112Y	0.01
112	S112R	0.01
112	S112P	0.01
112	S112H	0.38
112	S112V	0.48
112	S112M	0.56
112	S112W	0.58
112	S112K	0.68
112	S112T	0.72
112	S112N	0.85
112	S112F	0.88
112	S112A	0.94
113	V113S	0.57
113	V113G	0.58

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
113	V113K	0.72
113	V113H	0.76
113	V113W	0.80
113	V113L	0.85
113	V113T	0.86
113	V113D	0.87
113	V113E	0.94
113	V113C	0.94
113	V113F	0.96
113	V113Y	0.98
114	L114H	0.01
114	L114E	0.01
114	L114Q	0.12
114	L114P	0.28
114	L114S	0.55
114	L114V	0.60
114	L114N	0.77
115	V115I	0.99
116	T116Y	0.47
116	T116V	0.57
116	T116R	0.62
116	T116L	0.68
116	T116W	0.75
116	T116I	0.76
116	T116Q	0.77
116	T116P	0.84
116	T116G	0.90
116	T116E	0.91
116	T116A	0.95
116	T116S	0.96
117	Q117W	0.71
117	Q117V	0.76
117	Q117G	0.79
117	Q117S	0.87
118	V118K	0.01
118	V118W	0.01
118	V118E	0.01
118	V118R	0.07
118	V118P	0.22
118	V118D	0.40
118	V118I	0.55
118	V118G	0.56
118	V118S	0.82
118	V118A	0.85
118	V118T	0.92
118	V118M	0.93
118	V118F	1.00
119	L119G	0.01
119	L119S	0.01
119	L119F	0.01
119	L119R	0.01
119	L119P	0.01
119	L119T	0.10
119	L119N	0.11
119	L119V	0.15
119	L119W	0.20
119	L119C	0.24
119	L119D	0.28
119	L119E	0.32
119	L119I	0.43
119	L119H	0.46
119	L119Y	0.56
120	T120P	0.01
120	T120H	0.50
120	T120R	0.60
120	T120A	0.66
120	T120Q	0.78
120	T120C	0.92

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Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
121	S121P	0.38
121	S121R	0.70
121	S121W	0.77
121	S121K	0.78
121	S121G	0.99
122	A122G	0.01
122	A122D	0.06
122	A122F	0.15
122	A122H	0.17
122	A122R	0.40
122	A122S	0.43
122	A122K	0.45
122	A122E	0.47
122	A122T	0.52
122	A122P	0.55
122	A122I	0.65
122	A122N	0.70
122	A122Q	0.74
122	A122W	0.86
122	A122V	0.89
122	A122M	0.94
123	G123C	0.30
123	G123Q	0.31
123	G123T	0.54
123	G123E	0.56
123	G123V	0.59
123	G123R	0.60
123	G123N	0.71
123	G123H	0.74
123	G123F	0.80
123	G123P	0.81
123	G123D	0.84
124	G124I	0.01
124	G124H	0.01
124	G124M	0.01
124	G124W	0.01
124	G124P	0.01
124	G124A	0.03
124	G124Q	0.21
124	G124T	0.32
124	G124V	0.33
124	G124R	0.41
124	G124L	0.54
124	G124S	0.56
124	G124Y	0.56
124	G124N	0.60
124	G124D	0.64
124	G124C	0.67
124	G124F	0.95
125	V125W	0.25
125	V125E	0.39
125	V125R	0.47
125	V125C	0.54
125	V125D	0.54
125	V125P	0.62
125	V125F	0.63
125	V125S	0.79
125	V125Y	0.81
125	V125A	0.93
125	V125I	0.94
126	G126I	0.01
126	G126V	0.18
126	G126Y	0.23
126	G126L	0.54
126	G126A	0.55
126	G126E	0.60
126	G126P	0.67
126	G126T	0.74

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
126	G126R	0.76
126	G126N	0.85
126	G126S	0.90
126	G126C	0.98
127	T127L	0.01
127	T127E	0.01
127	T127Q	0.15
127	T127I	0.20
127	T127H	0.60
127	T127D	0.62
127	T127M	0.64
127	T127C	0.65
127	T127V	0.68
127	T127G	0.71
127	T127P	0.77
127	T127S	0.83
128	T128D	0.66
129	Y129W	0.01
129	Y129G	0.01
129	Y129K	0.01
129	Y129V	0.01
129	Y129T	0.14
129	Y129A	0.17
129	Y129R	0.18
129	Y129M	0.21
129	Y129D	0.23
129	Y129L	0.27
129	Y129N	0.53
129	Y129P	0.59
129	Y129C	0.61
129	Y129S	0.69
129	Y129F	0.71
130	P130T	0.01
130	P130H	0.01
130	P130G	0.01
130	P130S	0.01
130	P130L	0.09
130	P130E	0.22
130	P130W	0.28
130	P130V	0.37
130	P130I	0.41
130	P130A	0.44
130	P130F	0.48
130	P130R	0.53
130	P130K	0.55
130	P130C	0.64
130	P130M	0.76
131	A131W	0.01
131	A131D	0.40
131	A131Y	0.48
131	A131L	0.59
131	A131S	0.68
131	A131P	0.71
131	A131Q	0.74
131	A131V	0.78
131	A131H	0.82
131	A131G	0.87
131	A131E	0.97
132	P132V	0.01
132	P132T	0.01
132	P132W	0.01
132	P132F	0.01
132	P132I	0.01
132	P132H	0.01
132	P132R	0.01
132	P132D	0.01
133	K133C	0.01
133	K133A	0.10

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
133	K133V	0.23
133	K133G	0.31
133	K133H	0.31
133	K133M	0.33
133	K133T	0.39
133	K133I	0.45
133	K133Q	0.52
133	K133S	0.58
133	K133F	0.59
133	K133P	0.71
133	K133E	0.76
133	K133R	0.83
133	K133W	0.99
134	V134Q	0.79
134	V134T	0.86
134	V134I	0.89
135	L135T	0.01
135	L135W	0.01
135	L135K	0.01
135	L135S	0.01
135	L135F	0.01
135	L135G	0.01
135	L135R	0.01
135	L135P	0.01
135	L135Q	0.17
135	L135V	0.43
135	L135E	0.63
135	L135M	0.78
136	V136P	0.01
136	V136E	0.20
136	V136N	0.40
137	V137N	0.01
137	V137G	0.26
137	V137S	0.29
137	V137I	0.70
137	V137T	0.93
138	S138I	0.35
138	S138V	0.69
139	P139S	0.01
139	P139G	0.01
139	P139R	0.01
139	P139C	0.01
139	P139D	0.01
139	P139E	0.01
139	P139F	0.01
139	P139H	0.01
139	P139I	0.01
139	P139K	0.01
139	P139N	0.01
139	P139Q	0.01
139	P139T	0.01
139	P139V	0.01
140	P140T	0.01
140	P140S	0.01
140	P140V	0.01
140	P140W	0.01
140	P140I	0.01
140	P140Y	0.01
140	P140Q	0.01
140	P140R	0.01
141	P141R	0.01
141	P141G	0.01
141	P141S	0.02
141	P141T	0.12
141	P141V	0.16
141	P141Q	0.37
141	P141I	0.38
141	P141L	0.65

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
141	P141H	0.79
141	P141N	0.97
142	L142W	0.01
142	L142I	0.28
142	L142S	0.31
142	L142Q	0.33
142	L142V	0.33
142	L142P	0.44
142	L142F	0.54
142	L142A	0.56
142	L142K	0.66
142	L142C	0.70
143	A143W	0.01
143	A143P	0.39
143	A143G	0.42
143	A143S	0.63
143	A143F	0.68
143	A143Q	0.81
143	A143N	0.82
143	A143T	0.97
143	A143R	0.99
143	A143V	0.99
144	P144G	0.62
144	P144A	0.79
144	P144T	0.81
144	P144S	0.92
145	M145W	0.01
145	M145G	0.26
145	M145E	0.48
145	M145I	0.53
145	M145Q	0.57
145	M145L	0.61
145	M145V	0.63
145	M145R	0.69
145	M145F	0.77
145	M145P	0.78
145	M145S	0.78
145	M145T	0.79
145	M145A	0.79
145	M145Y	0.82
145	M145C	0.93
146	P146W	0.68
146	P146T	0.76
146	P146V	0.77
146	P146S	0.96
147	H147S	0.75
147	H147T	0.84
147	H147I	0.92
147	H147V	0.92
147	H147R	0.94
147	H147A	0.98
148	P148Q	0.98
149	W149R	0.01
149	W149E	0.01
149	W149P	0.01
149	W149C	0.12
149	W149I	0.24
149	W149A	0.31
149	W149S	0.33
149	W149Q	0.40
149	W149T	0.44
149	W149G	0.45
149	W149M	0.49
149	W149F	0.50
149	W149L	0.64
149	W149Y	0.75
150	F150P	0.32
150	F150N	0.36

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
150	F150G	0.46
150	F150V	0.51
150	F150A	0.54
150	F150T	0.58
150	F150W	0.62
150	F150M	0.63
150	F150E	0.73
150	F150C	0.78
150	F150I	0.78
150	F150K	0.85
151	Q151L	0.01
151	Q151V	0.01
151	Q151F	0.01
151	Q151I	0.01
151	Q151W	0.32
152	L152I	0.61
152	L152P	0.61
152	L152T	0.69
152	L152Q	0.76
152	L152G	0.77
152	L152S	0.84
152	L152D	0.86
152	L152V	0.88
152	L152R	0.91
152	L152K	0.91
152	L152H	0.92
153	I153N	0.89
154	F154T	0.01
154	F154G	0.01
154	F154V	0.01
154	F154S	0.29
154	F154Q	0.97
155	E155R	0.01
155	E155F	0.23

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
155	E155V	0.47
155	E155I	0.65
155	E155Q	0.69
156	G156I	0.01
156	G156F	0.73
156	G156W	0.90
156	G156L	0.94
156	G156V	0.97
157	G157R	0.01
157	G157P	0.01
157	G157S	0.19
157	G157V	0.40
157	G157C	0.61
157	G157E	0.84
157	G157M	0.85
157	G157A	0.87
157	G157D	0.94
157	G157T	0.99
158	E158V	0.89
158	E158D	0.89
158	E158T	0.91
158	E158I	0.94
159	Q159A	0.28
159	Q159C	0.31
159	Q159P	0.49
159	Q159D	0.63
159	Q159L	0.70
159	Q159G	0.72
159	Q159S	0.73
159	Q159R	0.74
159	Q159M	0.84
159	Q159E	0.97
160	K160W	0.01
160	K160G	0.30

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
160	K160H	0.57
160	K160S	0.70
160	K160L	0.95
160	K160I	1.00
161	T161R	0.01
161	T161H	0.01
161	T161W	0.01
161	T161N	0.01
161	T161G	0.43
161	T161C	0.56
161	T161S	0.57
161	T161I	0.98
163	E163F	0.27
163	E163R	0.49
163	E163V	0.55
163	E163P	0.77
163	E163G	0.80
163	E163H	0.82
163	E163S	0.85
163	E163W	0.98
164	L164Y	0.01
164	L164A	0.01
164	L164D	0.01
164	L164E	0.01
164	L164G	0.01
164	L164H	0.12
164	L164F	0.86
164	L164C	0.91
164	L164T	0.99
165	A165I	0.59
165	A165K	0.82
165	A165Y	0.84
165	A165S	0.94
165	A165F	1.00

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
166	R166T	0.74
166	R166V	0.76
166	R166G	0.91
166	R166S	0.95
168	Y168G	0.01
168	Y168T	0.01
168	Y168V	0.01
168	Y168I	0.01
168	Y168C	0.01
168	Y168Q	0.01
169	S169P	0.89
169	S169T	0.97
170	A170I	0.44
170	A170S	0.47
170	A170G	0.62
170	A170T	0.72
170	A170V	0.74
170	A170K	0.83
170	A170W	0.83
170	A170L	0.85
170	A170Q	0.89
170	A170Y	0.89
171	L171R	0.01
172	A172K	0.01
172	A172R	0.01
172	A172E	0.01
172	A172Q	0.18
172	A172V	0.39
172	A172W	0.45
172	A172P	0.58
172	A172I	0.58
172	A172T	0.71
172	A172N	0.76
172	A172G	0.84

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
172	A172S	0.85
172	A172C	0.86
174	F174W	0.01
174	F174Q	0.46
174	F174C	0.48
174	F174R	0.52
174	F174S	0.61
174	F174T	0.64
174	F174V	0.67
174	F174G	0.91
175	M175P	0.08
175	M175A	0.66
175	M175Y	0.72
175	M175G	0.75
175	M175W	0.76
175	M175V	0.81
175	M175Q	0.83
175	M175L	0.86
175	M175R	0.86
175	M175T	0.90
176	K176S	0.72
176	K176G	0.73
176	K176P	0.78
176	K176L	0.92
176	K176Y	0.93
176	K176N	0.94
176	K176T	0.97
176	K176Q	0.97
178	P178W	0.02
179	F179Q	0.01
179	F179S	0.34
179	F179W	0.86
179	F179H	0.93
179	F179N	0.95
180	F180K	0.01
180	F180T	0.01
180	F180R	0.01
180	F180S	0.01
180	F180G	0.01
180	F180Q	0.01
181	D181Y	0.01
181	D181W	0.01
181	D181L	0.01
181	D181T	0.01
181	D181V	0.01
181	D181R	0.22
181	D181K	0.47
181	D181G	0.52
181	D181S	0.55
181	D181Q	0.60
181	D181P	0.66
181	D181E	0.72
181	D181C	0.85
182	A182I	0.01
182	A182R	0.01
182	A182Q	0.01
182	A182P	0.01
182	A182T	0.11
182	A182N	0.53
182	A182S	0.85
182	A182G	0.94
182	A182C	0.99
183	G183S	0.01
183	G183Q	0.01
183	G183V	0.01
183	G183F	0.19
183	G183H	0.95
183	G183D	0.99

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
184	S184T	0.60
184	S184H	0.74
184	S184G	0.82
184	S184P	0.85
185	V185W	0.01
185	V185H	0.01
185	V185G	0.01
185	V185D	0.01
185	V185S	0.53
185	V185Y	0.58
185	V185I	0.63
185	V185R	0.79
185	V185K	0.79
185	V185C	0.83
185	V185E	0.88
185	V185T	0.91
185	V185L	0.93
186	I186G	0.01
186	I186S	0.01
186	I186R	0.01
186	I186P	0.01
186	I186T	0.23
186	I186V	0.48
186	I186F	0.76
187	S187P	0.01
187	S187T	0.23
187	S187Q	0.35
187	S187W	0.52
187	S187R	0.55
187	S187V	0.58
187	S187F	0.65
187	S187Y	0.80
188	T188H	0.01
188	T188R	0.01

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
188	T188F	0.01
188	T188Y	0.09
188	T188I	0.10
188	T188V	0.15
188	T188L	0.42
188	T188M	0.75
188	T188G	0.79
188	T188C	0.87
188	T188S	0.91
188	T188A	0.95
189	D189F	0.37
189	D189R	0.39
189	D189N	0.57
189	D189V	0.71
189	D189W	0.76
189	D189E	0.77
189	D189G	0.80
189	D189S	0.81
189	D189M	0.88
189	D189C	0.94
189	D189H	0.95
189	D189P	0.97
190	G190V	0.01
190	G190S	0.01
190	G190Q	0.29
190	G190W	0.41
190	G190R	0.51
190	G190K	0.57
190	G190L	0.82
191	V191H	0.01
191	V191W	0.01
191	V191S	0.01
191	V191G	0.01
191	V191N	0.01

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
191	V191I	0.02
192	D192S	0.01
192	D192P	0.01
192	D192F	0.01
192	D192H	0.01
192	D192I	0.01
192	D192Q	0.01
192	D192R	0.01
192	D192T	0.01
192	D192V	0.01
192	D192W	0.01
192	D192N	0.15
192	D192C	0.56
193	G193H	0.01
193	G193C	0.01
193	G193T	0.01
193	G193N	0.01
194	I194S	0.01
194	I194A	0.01
194	I194C	0.01
194	I194P	0.01
194	I194F	0.01
194	I194W	0.01
194	I194R	0.01
194	I194Y	0.01
194	I194G	0.04
194	I194L	0.58
194	I194V	0.78
195	H195S	0.08
195	H195C	0.10
195	H195L	0.18
195	H195N	0.22
195	H195R	0.24
195	H195F	0.40

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
195	H195V	0.60
195	H195Q	0.96
195	H195A	0.98
196	F196H	0.01
196	F196G	0.01
196	F196S	0.01
196	F196Q	0.01
196	F196W	0.38
196	F196P	0.39
196	F196V	0.68
196	F196M	0.71
196	F196Y	0.97
197	T197R	0.01
197	T197L	0.65
197	T197S	0.75
197	T197G	0.81
197	T197I	0.84
197	T197C	0.86
197	T197V	0.89
197	T197N	0.91
199	A199M	0.93
199	A199S	0.99
199	A199G	0.99
201	N201Y	0.01
201	N201T	0.01
201	N201V	0.01
201	N201R	0.01
201	N201S	0.06
201	N201H	0.10
201	N201G	0.30
201	N201L	0.35
201	N201F	0.67
201	N201E	0.72
203	D203V	0.50

Table 10-7. Variants with Peracid Degradation Results Less than Wild-Type

Pos	WT/Pos./Var.	PAD PI
203	D203W	0.52
203	D203E	0.90

The following Table provides variants that have protein performance indices ("Prot. PI") better than wild-type.

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
2	A002Y	1.61
2	A002N	1.30
2	A002I	1.25
2	A002V	1.18
2	A002T	1.17
2	A002S	1.15
5	I005M	1.29
7	C007A	1.22
7	C007G	1.07
7	C007M	1.03
8	F008N	1.23
8	F008M	1.05
8	F008G	1.03
8	F008P	1.01
11	S011H	1.06
11	S011A	1.04
11	S011D	1.03
11	S011E	1.01
11	S011Q	1.01
12	L012N	1.06
12	L012Q	1.05
13	T013V	1.17
14	W014Y	1.02
16	W016Y	1.02
17	V017A	1.21
17	V017E	1.11
17	V017F	1.09
17	V017I	1.08
17	V017K	1.06
17	V017T	1.03
18	P018C	2.56
18	P018H	2.50
18	P018L	2.50
18	P018E	2.47
18	P018G	2.47
18	P018N	2.35
18	P018V	2.30
18	P018Q	2.13
18	P018R	2.01
18	P018Y	1.68
18	P018S	1.05
19	V019G	1.39
19	V019A	1.23
19	V019E	1.10
19	V019Q	1.07
19	V019K	1.03
19	V019M	1.00
20	E020G	1.11

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Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
20	E020P	1.08
20	E020A	1.08
20	E020N	1.01
20	E020V	1.01
22	G022A	1.07
22	G022I	1.03
23	A023F	1.03
24	P024T	1.43
24	P024G	1.34
24	P024S	1.31
24	P024H	1.15
24	P024I	1.11
24	P024L	1.06
25	T025C	1.37
25	T025V	1.30
25	T025G	1.27
25	T025A	1.23
25	T025I	1.19
25	T025P	1.10
25	T025M	1.04
29	A029G	1.22
29	A029P	1.07
29	A029M	1.06
29	A029D	1.06
29	A029V	1.05
29	A029S	1.05
29	A029T	1.02
29	A029E	1.02
30	P030E	1.20
30	P030A	1.15
30	P030S	1.12
30	P030L	1.07
30	P030Q	1.06
30	P030K	1.06

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
30	P030H	1.05
30	P030Y	1.04
32	V032M	1.11
32	V032A	1.10
32	V032I	1.08
32	V032Q	1.03
32	V032L	1.01
35	T035C	1.16
36	G036C	1.09
36	G036N	1.08
36	G036Q	1.07
36	G036S	1.06
36	G036A	1.00
37	V037N	1.09
39	A039V	1.18
39	A039E	1.03
46	F046A	1.05
46	F046C	1.01
47	E047I	1.02
54	S054A	1.33
54	S054C	1.21
54	S054E	1.16
54	S054D	1.08
54	S054H	1.06
54	S054N	1.01
54	S054M	1.01
55	A055N	1.12
55	A055S	1.08
56	R056Q	1.02
58	T058V	1.13
60	I060A	1.20
60	I060M	1.14
60	I060V	1.06
60	I060L	1.02

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
61	D061A	1.41
61	D061N	1.12
61	D061V	1.10
61	D061Y	1.03
61	D061Q	1.02
61	D061L	1.00
62	D062A	1.06
62	D062M	1.06
63	P063S	1.17
63	P063Y	1.12
63	P063M	1.09
63	P063Q	1.08
63	P063A	1.06
63	P063V	1.06
63	P063R	1.02
63	P063T	1.02
64	T064Q	1.13
64	T064M	1.07
64	T064R	1.05
64	T064C	1.05
64	T064S	1.03
66	P066Q	1.91
66	P066G	1.78
66	P066N	1.62
66	P066C	1.51
66	P066I	1.51
66	P066R	1.26
66	P066H	1.23
66	P066V	1.12
66	P066Y	1.08
66	P066A	1.03
66	P066F	1.02
67	R067Q	1.60
67	R067L	1.46

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
67	R067A	1.39
67	R067V	1.24
67	R067P	1.04
67	R067F	1.01
68	L068A	1.07
68	L068V	1.01
68	L068G	1.00
69	N069C	1.18
69	N069G	1.06
69	N069D	1.05
69	N069S	1.03
70	G070A	1.08
72	S072L	1.07
72	S072A	1.06
72	S072Y	1.03
73	Y073N	1.25
73	Y073Q	1.20
73	Y073C	1.18
73	Y073D	1.09
73	Y073V	1.08
73	Y073M	1.05
73	Y073L	1.03
74	L074I	1.45
74	L074Y	1.19
74	L074V	1.18
74	L074A	1.01
75	P075M	1.22
75	P075S	1.18
75	P075T	1.10
75	P075Y	1.08
75	P075C	1.06
75	P075Q	1.04
75	P075L	1.02
75	P075E	1.00

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Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
76	S076W	1.06
77	C077L	1.44
77	C077V	1.33
77	C077A	1.20
77	C077S	1.19
77	C077T	1.18
78	L078I	1.06
78	L078V	1.04
79	A079C	1.16
79	A079E	1.12
79	A079S	1.09
79	A079Q	1.05
79	A079M	1.04
79	A079R	1.02
80	T080S	1.12
80	T080E	1.02
80	T080Q	1.02
82	L082G	1.24
82	L082R	1.15
82	L082V	1.14
82	L082S	1.13
82	L082P	1.11
82	L082M	1.07
82	L082K	1.03
82	L082A	1.00
83	P083G	1.01
84	L084V	1.23
86	L086Q	3.66
89	I089V	1.09
89	I089L	1.07
93	T093Q	2.03
96	T096A	1.32
96	T096V	1.12
96	T096S	1.05
96	T096G	1.03
97	K097A	1.11
97	K097R	1.02
98	A098S	1.17
98	A098T	1.03
98	A098N	1.01
99	Y099S	1.45
99	Y099L	1.39
99	Y099H	1.30
99	Y099A	1.29
99	Y099V	1.28
99	Y099G	1.23
99	Y099W	1.20
99	Y099I	1.11
100	F100M	1.20
100	F100N	1.12
100	F100W	1.06
100	F100S	1.02
101	R101L	1.33
101	R101N	1.11
101	R101Q	1.03
101	R101D	1.02
102	R102Q	1.09
103	T103G	1.20
103	T103S	1.14
103	T103H	1.14
103	T103N	1.07
103	T103K	1.05
103	T103P	1.01
104	P104S	1.44
104	P104V	1.40
104	P104E	1.37
104	P104C	1.34
104	P104N	1.32

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
104	P104T	1.29
104	P104G	1.25
104	P104Q	1.24
104	P104H	1.11
104	P104I	1.07
104	P104M	1.01
105	L105Y	1.18
105	L105H	1.07
105	L105G	1.07
105	L105C	1.05
105	L105Q	1.03
105	L105T	1.00
105	L105P	1.00
106	D106E	1.02
107	I107S	1.05
107	I107V	1.04
107	I107C	1.00
108	A108G	1.15
108	A108S	1.14
108	A108T	1.08
109	L109E	1.24
109	L109I	1.21
109	L109D	1.15
109	L109N	1.13
109	L109F	1.11
109	L109Q	1.08
109	L109A	1.07
109	L109H	1.06
109	L109V	1.06
109	L109M	1.00
110	G110S	1.01
112	S112N	1.09
112	S112E	1.05
113	V113C	1.06
113	V113N	1.01
114	L114C	1.10
114	L114A	1.03
114	L114M	1.00
115	V115I	1.14
115	V115C	1.14
115	V115A	1.11
115	V115M	1.05
115	V115L	1.02
116	T116N	1.68
116	T116H	1.48
116	T116G	1.44
116	T116C	1.30
116	T116E	1.29
116	T116Q	1.29
116	T116M	1.28
116	T116S	1.24
116	T116Y	1.09
116	T116A	1.08
116	T116R	1.03
116	T116L	1.03
117	Q117S	1.13
117	Q117H	1.12
117	Q117E	1.10
117	Q117T	1.06
117	Q117A	1.03
118	V118C	1.28
118	V118A	1.20
118	V118I	1.01
119	L119C	1.18
119	L119A	1.18
119	L119N	1.14
119	L119I	1.06
119	L119S	1.05

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
119	L119V	1.04
119	L119E	1.04
119	L119R	1.00
120	T120S	1.35
120	T120E	1.19
120	T120C	1.14
120	T120K	1.12
120	T120N	1.10
120	T120A	1.09
120	T120H	1.07
120	T120Q	1.05
120	T120Y	1.01
120	T120L	1.00
121	S121N	1.17
121	S121L	1.12
121	S121A	1.10
121	S121C	1.09
121	S121G	1.07
121	S121R	1.06
121	S121K	1.04
121	S121E	1.01
121	S121Q	1.01
122	A122N	1.11
122	A122L	1.07
122	A122P	1.07
122	A122M	1.06
122	A122V	1.05
122	A122S	1.05
122	A122E	1.04
122	A122I	1.04
122	A122Q	1.02
124	G124M	1.36
124	G124A	1.20
124	G124N	1.18

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
124	G124C	1.07
124	G124Q	1.02
125	V125I	1.05
126	G126N	1.04
126	G126E	1.02
126	G126A	1.02
127	T127A	1.10
127	T127S	1.08
127	T127V	1.06
127	T127C	1.04
127	T127G	1.04
127	T127D	1.03
127	T127E	1.03
127	T127M	1.02
128	T128N	1.29
128	T128M	1.28
128	T128Q	1.24
128	T128A	1.23
128	T128H	1.19
128	T128P	1.18
128	T128D	1.14
128	T128K	1.10
128	T128S	1.07
128	T128V	1.05
128	T128R	1.03
128	T128F	1.01
129	Y129F	1.44
129	Y129C	1.42
129	Y129A	1.39
129	Y129D	1.35
129	Y129M	1.28
129	Y129N	1.24
129	Y129L	1.22
129	Y129P	1.11

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
129	Y129G	1.10
129	Y129S	1.08
129	Y129W	1.01
129	Y129V	1.00
130	P130G	1.11
130	P130E	1.08
130	P130K	1.05
130	P130A	1.03
130	P130M	1.03
133	K133Q	1.13
133	K133S	1.02
133	K133A	1.01
133	K133R	1.01
133	K133E	1.01
135	L135M	1.01
136	V136L	1.03
138	S138A	1.44
138	S138C	1.17
138	S138G	1.09
141	P141A	1.13
141	P141G	1.02
142	L142I	1.05
143	A143G	1.17
145	M145I	1.16
145	M145L	1.07
147	H147L	1.09
147	H147C	1.04
149	W149G	1.39
149	W149A	1.35
149	W149M	1.32
149	W149S	1.28
149	W149F	1.27
149	W149Y	1.15
149	W149Q	1.10
149	W149L	1.06
150	F150A	1.70
150	F150M	1.69
150	F150N	1.52
150	F150C	1.41
150	F150P	1.38
150	F150K	1.33
150	F150E	1.32
150	F150T	1.27
150	F150V	1.26
150	F150W	1.26
150	F150Y	1.24
150	F150I	1.19
150	F150L	1.14
150	F150G	1.13
150	F150H	1.09
151	Q151K	1.04
153	I153N	1.04
157	G157A	1.00
159	Q159E	1.14
159	Q159A	1.13
159	Q159G	1.03
161	T161C	1.01
162	T162C	1.17
162	T162I	1.16
162	T162H	1.08
162	T162L	1.05
162	T162F	1.05
162	T162Y	1.03
164	L164M	1.09
164	L164V	1.08
165	A165G	1.14
165	A165Q	1.05
165	A165S	1.05

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Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
166	R166M	1.26
166	R166K	1.19
166	R166G	1.19
166	R166N	1.16
166	R166D	1.16
166	R166A	1.12
166	R166L	1.08
166	R166T	1.04
167	V167L	1.13
167	V167H	1.12
167	V167G	1.08
167	V167M	1.04
167	V167I	1.04
167	V167S	1.04
167	V167C	1.01
168	Y168F	1.28
168	Y168L	1.27
170	A170C	1.02
171	L171I	1.16
172	A172C	1.09
172	A172G	1.07
175	M175Y	1.35
175	M175L	1.19
175	M175W	1.14
175	M175N	1.11
175	M175R	1.02
176	K176R	1.06
176	K176Q	1.02
178	P178E	1.05
182	A182C	1.03
183	G183S	1.08
184	S184E	1.39
184	S184A	1.31
184	S184M	1.25

Table 10-8. Sites with Protein PI Values Better Than Wild-Type

Pos	WT/Pos./Var.	Prot. PI
184	S184G	1.15
184	S184D	1.15
184	S184C	1.14
184	S184Q	1.09
184	S184H	1.07
184	S184N	1.03
184	S184V	1.03
184	S184K	1.02
185	V185I	1.03
186	I186M	1.11
188	T188C	2.04
188	T188I	1.85
188	T188L	1.76
188	T188M	1.60
188	T188V	1.53
188	T188S	1.52
188	T188R	1.41
188	T188A	1.40
188	T188G	1.32
188	T188N	1.24
191	V191C	1.04
194	I194L	1.32
194	I194C	1.17
194	I194A	1.15
194	I194W	1.12
194	I194V	1.03
194	I194Y	1.01
196	F196L	1.09
201	N201H	1.49

The following Table provides variants that have a PAD PI that is greater than 1.5, a PAF that is greater than or equal to 0.1, and a protein PI that is greater than or equal to 0.1

Table 10-9. PAD PI > 1.5 with PAF ≥ 0.1 and protein PI > 0.1

Wild-Type Amino Acid/ Pos.	Variant Amino Acid
M1	L
K3	A, C, H, I, L
R4	A
I5	A, C, E, L
L6	A
C7	K
T13	A, C
P18	C, E, G, H, L, Q, R, V, Y
E20	C, Q
D21	A, G, K, L, Y
G22	A
P24	L
E26	L
R27	A, K, L
F28	D, L
P30	T, V
D31	L, N
V32	A, D, E, G, I, K, L, M, N, Q, W
R33	C, G, K, L
T35	A, C, I, M
G36	K
Q40	D, G, K, S, T, W, Y
Q41	A, K, L
G43	E, L
A44	C
F46	L
V48	A, C, L, M, P
I49	A
E51	A
L53	H
N59	A, C, D, E, F, G, K, L, Q, S, T, V, W, Y
D61	I, K, R
N69	H, I, K, V
S72	A, C, G, H, M, N
P75	D, G, K, S, T, W, Y
S76	D, E, G, M
T80	G

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Table 10-9. PAD PI > 1.5 with PAF ≥ 0.1 and protein PI > 0.1

Wild-Type Amino Acid/ Pos.	Variant Amino Acid
H81	M
P83	A, M
D85	F, G
L86	C
V87	C, L
I89	A
T96	A, C, L, M
A98	D
F100	A, M
R102	A, L
P104	C, E, I, M
L105	C, F, W
D106	V
I107	T
G110	E, L
V115	G
Q117	A, M
V118	Q
T120	E, I, Y
S121	A, C, V
T128	F, K, L, R, Y
P132	A, C, E, G, L, Q, S, Y
K133	L
V134	A, M
V136	A
P140	A
P144	H, Y
P146	C, F, H, L
P148	F
Q151	A, C, D, E, H, K, P, R, S, T, Y
L152	W
I153	F, H, K, P, S, T
F154	Y
E155	A, L, M, N, P, Y
G156	D, M, T
G157	H
E158	F, K, L, M, N, Y
T161	M, Q
T162	C, F, G, H, I, K, L, M, N, P, Q, S, W, Y
E163	A, L, Y
A165	D, L, M
R166	A, D, H, L
V167	A, C, D,G, H, L, M, P, Q, R, S, T, Y
Y168	F, L
S169	I
L171	A, C, F, K, M, N, Q, S
S173	A, C, E, F, I, K, L, M, P, R, V, W, Y
F174	A, L, M, Y
P178	A, D, E, G, K, L, M, Q, S, T,

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Table 10-9. PAD PI > 1.5 with PAF ≥ 0.1 and protein PI ≥ 0.1

Wild-Type Amino Acid/ Pos.	Variant Amino Acid
	V, Y
F179	L
G190	A, H, M
V191	A, C, D, E, F, K, L, M, Q, R, Y
G193	S, V
T197	M
E198	C, L, M, N, P, R, W, Y
A199	C, K, L, Y
R202	A, C, E, F, G, H, I, L, M, S, T, W
D203	A, C, H, L, R
G205	A
V206	C, E, F, G, H, K, L, M, N, P, R
A209	E, L
E210	D, K
Q211	M, N, P
S214	A, C, D, F, G, I, K, L, R, T, V, W,
L215	E, M, T, V, Y

The following Table provides variants with a PAD PI that is less than 0.5, a PAF that is greater than or equal to 0.1, and a protein PI that is greater than or equal to 0.1.

Table 10-10. PAD PI < 0.5 with PAF >0.1, and Protein PI >0.1

Wild-Type Residue/Pos.	Amino Acid Variant(s)
A2	Y
R4	I, L, V
I5	S
L6	S, T, V
F8	R
D10	G
L12	A, C, F, G, K, Q, R, S, T, V
W14	F, G, I, K, L, R, S, T, V
G15	C, N
P18	S
V19	M, Q, R
G22	K, W
A23	G, R, S,
T25	G, H, I, K, L, M, P, R, W
E26	N, S, T, W
R27	P, T, W
F28	G
A29	T, V
T35	N, Q, V
G36	S, T
L38	G, S
Q41	S, V
L42	Q, S, T
G43	P, Q, S, V
D45	R, S, T
F46	T
E47	P
V48	S
I49	P, R
E50	V
E51	I, V
G52	H, L, S, V
L53	E, G, K, R, S
S54	F, G, I, K, L, R, T, V,W, Y
A55	I, R, T, V
R56	C, G, S, T
T57	C, N
T58	A, M
N59	M, R
I60	P
D62	C, G, H, I, L, R, S, T, V, W
T64	R
D65	H, R, S, V, Y
P66	G, N, Q
R67	E, F, G, L, N, P, Q, T, V, W
L68	A, C, E, F, G, H, M, N, P, Q, R, S, T, Y
N69	Y
G70	C, T
S72	W, Y
Y73	L, R
P75	M, R

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Table 10-10. PAD PI < 0.5 with PAF >0.1, and Protein PI >0.1

Wild-Type Residue/Pos.	Amino Acid Variant(s)
S76	F, W, Y
C77	F, W, Y
L78	M
A79	C, E, H, M, N, Q, R
T80	H, I, K, L, W, Y
H81	R, Y
L82	G, H, R, S, T, V, W
P83	T, V
L84	A, T, V, W
D85	I, L, V, W
L86	H, S, T, V, W
V87	A, F, G, S, T, Y
I88	T, V
I89	S
M90	S, T, V
L91	T, V
T93	S, Y
N94	H, L, T, V
T96	I, R, W, Y
K97	G,I, L, P, Q, S, T, V, Y
A98	T
Y99	S, V
F100	E, K, W
R101	K, Q, V, W
R102	C, G
T103	A, C, F, G, H, I, K, L, N, P, R, S, V, W, Y
P104	R, T
L105	V
I107	P, Q
L109	A, D, E, F, H, I, Q, R, S, W
G110	Q, S, T
M111	G, H, R, S
S112	H, R, V, Y
L114	Q
T116	Y
V118	P, R, W
L119	C, D, E, F, G, H, I, N, R, S, T, V, W
T120	H
S121	P
A122	D, E, F, G, H, K, R, S
G123	C
G124	A, H, I, M, Q, R, T, V, W
V125	E, R, W
G126	I, V, Y
T127	E, I, L, Q
Y129	A, D, G, K, L, M, R, T, V, W
P130	A, E, F, G, H, I, L, S, T, V, W
A131	D, W, Y
P132	F, H, I, T, V
K133	A, C, G, H, I, M, T, V
L135	F, Q, S, T, V
V137	S
S138	I
P139	S
P140	S
P141	G, I, Q, R, S,T, V

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Table 10-10. PAD PI < 0.5 with PAF ≥0.1, and Protein PI ≥0.1	
Wild-Type Residue/Pos.	**Amino Acid Variant(s)**
L142	Q, S, V
A143	G, P, W
M145	E, G, W
W149	A, C, F, G, I, M, Q, S, T
F150	G, N, P, W
E155	F, R, V
G156	I
G157	R, S, V
Q159	A, C, P
K160	G
T161	G, H, R, W
E163	F, R
Y168	C, I, V
A170	I, S
A172	Q, V
F174	C, Q, W
F179	Q, S
G190	S, V, W
V191	G, H, I, N, S, W
G193	C, H, T
I194	A, C, G, S
F196	G, Q, W
T197	R
N201	G, H, L, R, S, T, V, Y
D203	V
L208	Q, S, V, Y
V212	G
L215	A, C, G, K, P, R
L216	G, I, T

In addition to the assay results described above, various mutations were found to result in unstable protein such that perhydrolase protein was not expressed. Thus, in contrast to the substitutions that resulted in enhanced expression as compared to wild-type, there were some substitutions that are not as favorable, at least under the conditions used herein. However, it is not intended that the present invention exclude these substitutions, as it is contemplated that these substitutions, taken alone or in combination will find use in alternative embodiments of the present invention.

Table 10-11. Mutations that Produced Unstable Protein

Wild-Type/Pos.	Variant Amino Acid
M1	A, E, F, G, K, N, P, R, S,T,W
I5	W
C7	L, P, T, W
G9	A, C, E, K, L, P, Q, R, V
T13	F, R, W
G15	H, K, L, R, Y
P18	A
D21	V
F28	H, I, R
R33	D,E, H, P, W
W34	K
T35	K, L, P, W, Y
G36	P
V37	Q, R
L38	W
A39	F
L42	D
A44	D, H, P
F46	H
V48	W
E51	P
R56	H, K, P, W, Y
T57	W
T58	E, G, K, P, R, W, Y
L74	D, H, P, Q, R, T
C77	N, P
L78	A, P, R, S
A79	V
L86	F
I88	R, Y
I89	D, R
L91	H, K, P, R, W, Y
G92	A, D, L, M, P, R, T, W, Y
T93	P, R, V, W
D95	A, D, G, H, K, L, N, Q, R, S, T, V, W, Y
K97	D
P104	A, L

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Table 10-11. Mutations that Produced Unstable Protein

Wild-Type/Pos.	Variant Amino Acid
L105	A, M
I107	H, W
A108	D, F, H, I, N, P, R
G110	L
L114	F, K, R, W, Y
V115	H, K,
V134	D, K, R, W, Y
V136	R, W
V137	D, E, F, P, R, W
S138	E, F, H, L,M, Q, R, W, Y
P139	L, W, Y
P140	D, K, L, M
L142	D, G, M, N, R, T
H147	G
F154	E, L, P,
T161	D, E, P
Y168	D, E, H, K, N, P, R, S, W
L171	D
F179	A, P, R
F180	E
D181	F, H, I, M, N
A182	H, K, L, M, W, Y
I186	K, W, Y
T188	D, K, P, Q, W
F196	A, K, N, R

The following Table provides performance indices obtained in PAF and PAD assays for various variants, as well as the protein performance index.

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
M1	A	-0.12	-0.12	-0.01
M1	E	-0.12	-0.12	-0.01
M1	F	-0.12	-0.12	-0.01
M1	G	-0.12	-0.12	-0.01
M1	I	0.96	1.19	0.31
M1	K	-0.12	-0.12	-0.01
M1	L	0.75	2.11	0.30
M1	M	1.00	1.00	1.00
M1	N	-0.12	-0.12	-0.01
M1	P	-0.12	-0.12	-0.01
M1	R	-0.12	-0.12	-0.01
M1	S	-0.12	-0.12	-0.01
M1	T	-0.12	-0.12	-0.01
M1	V	0.87	0.94	0.52
M1	W	-0.12	-0.12	-0.01
A2	A	1.00	1.00	1.00
A2	D	1.30	1.05	0.77
A2	E	0.61	1.38	0.52
A2	F	1.24	0.93	0.89
A2	G	1.15	0.84	0.95
A2	I	1.18	0.61	1.25
A2	N	0.93	0.59	1.30
A2	P	0.52	1.17	0.68
A2	Q	0.81	1.29	0.65
A2	R	0.90	1.17	0.70
A2	S	1.01	0.66	1.15
A2	T	0.98	0.61	1.17
A2	V	0.89	0.60	1.18
A2	W	1.75	1.17	0.53
A2	Y	0.84	0.46	1.61
K3	A	0.86	2.14	0.48
K3	C	0.81	1.52	0.67
K3	E	0.12	3.51	0.11
K3	G	0.72	3.74	0.08
K3	H	1.01	1.89	0.30
K3	I	1.05	2.44	0.16

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
K3	K	1.00	1.00	1.00
K3	L	1.04	1.84	0.50
K3	M	0.85	1.44	0.71
K3	P	0.80	1.45	0.59
K3	Q	0.87	1.19	0.69
K3	R	0.87	1.29	0.46
K3	S	0.94	1.17	0.44
K3	T	1.01	1.03	0.71
K3	V	0.81	0.84	0.33
K3	Y	1.06	1.39	0.86
R4	A	0.41	1.64	0.29
R4	C	0.71	1.34	0.35
R4	D	0.27	1.18	0.32
R4	E	0.32	0.97	0.25
R4	G	0.79	0.79	0.41
R4	H	0.92	0.99	0.59
R4	I	0.24	0.15	0.18
R4	L	0.21	-0.03	0.18
R4	P	0.14	1.44	0.13
R4	Q	1.03	0.99	0.70
R4	R	1.00	1.00	1.00
R4	S	0.65	0.91	0.64
R4	T	0.80	1.00	0.69
R4	V	0.29	0.08	0.22
R4	W	0.04	0.48	0.12
R4	Y	0.63	0.98	0.39
I5	A	0.60	1.88	0.62
I5	C	0.44	2.47	0.54
I5	D	-0.13	3.11	0.06
I5	E	0.67	1.59	0.33
I5	F	-0.13	0.15	0.06
I5	G	0.05	-3.88	0.10
I5	H	0.55	0.63	0.18
I5	I	1.00	1.00	1.00
I5	L	0.80	1.63	0.96
I5	M	0.63	1.09	1.29

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
I5	N	-0.13	-2.15	0.12
I5	P	-0.13	-0.86	0.08
I5	R	-0.13	-6.48	0.08
I5	S	1.02	0.37	0.39
I5	T	1.12	0.72	0.25
I5	V	0.94	0.92	0.54
I5	W	-0.13	-0.44	-0.01
L6	A	0.87	1.99	0.26
L6	C	0.85	1.22	0.55
L6	E	-0.20	-0.59	0.09
L6	G	0.23	-3.45	0.12
L6	H	0.23	-1.08	0.09
L6	I	1.07	0.82	0.86
L6	K	0.41	-1.16	0.05
L6	L	1.00	1.00	1.00
L6	M	0.92	1.44	0.63
L6	Q	-0.20	-1.63	0.12
L6	R	0.06	-1.59	0.12
L6	S	0.58	-1.26	0.23
L6	T	1.06	0.35	0.40
L6	V	1.07	0.35	0.44
L6	W	0.06	-2.97	0.09
C7	A	1.42	1.03	1.22
C7	C	1.00	1.00	1.00
C7	E	-0.26	1.63	0.20
C7	G	1.39	0.69	1.07
C7	H	1.73	1.37	0.41
C7	I	1.76	1.48	0.31
C7	K	2.69	2.95	0.21
C7	L	-0.26	-0.16	-0.01
C7	M	1.13	0.68	1.03
C7	P	-0.26	-0.16	-0.01
C7	R	0.22	-1.04	0.15
C7	S	0.62	-2.83	0.10
C7	T	-0.26	-0.16	-0.01
C7	W	-0.26	-0.16	-0.01

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
C7	Y	2.09	0.54	0.67
F8	A	0.55	1.33	0.96
F8	C	-0.11	4.01	0.10
F8	F	1.00	1.00	1.00
F8	G	1.09	0.65	1.03
F8	H	1.02	0.64	0.97
F8	K	0.81	0.83	0.95
F8	L	0.77	1.31	0.90
F8	M	0.56	1.11	1.05
F8	N	-0.11	0.96	1.23
F8	P	1.00	0.83	1.01
F8	R	1.43	0.46	0.73
F8	S	0.71	-2.75	0.13
F8	T	0.88	0.77	0.94
F8	V	1.18	0.85	0.88
F8	Y	0.96	0.90	0.85
G9	A	-0.15	-0.18	-0.01
G9	C	-0.15	-0.18	-0.01
G9	E	-0.15	-0.18	-0.01
G9	G	1.00	1.00	1.00
G9	H	0.29	-0.06	0.16
G9	K	-0.15	-0.18	-0.01
G9	L	-0.15	-0.18	-0.01
G9	P	-0.15	-0.18	-0.01
G9	Q	-0.15	-0.18	-0.01
G9	R	-0.15	-0.18	-0.01
G9	T	0.21	-2.56	0.12
G9	V	-0.15	-0.18	-0.01
D10	A	-0.29	-14.24	0.02
D10	D	1.00	1.00	1.00
D10	E	0.01	0.15	0.72
D10	G	0.41	-0.92	0.17
D10	I	1.28	-6.86	0.04
D10	K	2.13	-5.30	0.02
D10	L	3.97	2.04	0.02
D10	M	-0.29	-5.94	0.04

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
D10	N	-0.29	-2.23	0.07
D10	P	-0.29	-4.16	0.05
D10	R	0.22	-4.36	0.06
D10	S	0.79	-0.58	0.06
D10	T	1.47	-0.45	0.06
D10	V	0.98	-4.22	0.06
D10	W	3.18	-3.70	0.02
D10	Y	1.51	-4.97	0.03
S11	A	0.25	0.53	1.04
S11	D	-0.25	-0.22	1.03
S11	E	-0.25	-0.23	1.01
S11	F	-0.25	-0.13	0.68
S11	G	-0.25	-0.09	0.86
S11	H	-0.25	0.33	1.06
S11	I	-0.25	0.56	0.63
S11	K	-0.25	0.40	0.62
S11	L	-0.25	-0.22	0.68
S11	Q	-0.25	-0.26	1.01
S11	R	-0.25	-0.08	0.69
S11	S	1.00	1.00	1.00
S11	T	0.04	-0.36	0.87
S11	V	0.03	-0.15	0.59
L12	A	1.10	0.07	0.71
L12	C	2.29	0.22	0.81
L12	D	0.04	0.00	0.39
L12	F	0.13	0.17	0.60
L12	G	0.44	-0.06	0.60
L12	H	0.02	0.16	0.77
L12	K	0.18	0.13	0.40
L12	L	1.00	1.00	1.00
L12	N	0.53	0.66	1.06
L12	P	0.03	-0.16	0.31
L12	Q	2.65	0.22	1.05
L12	R	0.23	-0.02	0.34
L12	S	0.54	-0.07	0.80
L12	T	0.68	0.06	0.89

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L12	V	0.98	-0.05	0.51
L12	W	0.03	0.02	0.33
T13	A	0.25	1.88	0.72
T13	C	0.56	1.55	0.78
T13	E	-0.10	1.09	0.44
T13	F	-0.10	-0.11	-0.02
T13	G	0.32	0.77	0.57
T13	I	0.12	1.05	0.69
T13	L	0.55	1.47	0.76
T13	M	0.17	1.47	0.94
T13	N	-0.10	2.61	0.27
T13	P	-0.10	2.73	0.17
T13	Q	0.01	0.51	0.98
T13	R	-0.10	-0.11	-0.02
T13	S	0.73	0.68	0.88
T13	T	1.00	1.00	1.00
T13	V	0.19	0.63	1.17
T13	W	-0.10	-0.11	-0.02
W14	A	-0.23	0.27	0.94
W14	E	-0.06	0.15	0.80
W14	F	0.29	0.22	0.71
W14	G	0.30	-0.97	0.70
W14	I	0.33	-0.42	0.66
W14	K	0.29	-0.17	0.71
W14	L	0.25	-0.36	0.82
W14	N	-0.23	-0.12	0.81
W14	P	-0.23	-0.29	0.34
W14	R	0.23	-0.40	0.66
W14	S	0.31	-0.99	0.69
W14	T	0.24	-0.77	0.64
W14	V	0.26	-0.49	0.58
W14	W	1.00	1.00	1.00
W14	Y	0.31	0.66	1.02
G15	A	1.54	0.61	0.87
G15	C	0.71	-0.27	0.66
G15	D	-0.18	0.01	0.26

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
G15	E	-0.18	-1.42	0.11
G15	G	1.00	1.00	1.00
G15	H	-0.18	-0.14	-0.01
G15	K	-0.18	-0.14	-0.01
G15	L	-0.18	-0.14	-0.01
G15	N	0.46	-0.63	0.71
G15	P	-0.18	-5.42	0.09
G15	R	-0.18	-0.14	-0.01
G15	S	1.05	0.63	0.76
G15	Y	-0.18	-0.14	-0.01
W16	A	0.12	0.55	0.50
W16	D	0.02	0.57	0.32
W16	E	0.06	0.65	0.46
W16	G	0.05	-0.07	0.38
W16	H	0.03	-0.02	0.55
W16	I	0.02	1.06	0.74
W16	K	0.01	1.03	0.73
W16	L	-0.48	1.16	0.76
W16	M	0.04	0.37	0.56
W16	N	0.02	-0.03	0.43
W16	P	0.03	0.15	0.37
W16	Q	0.05	0.31	0.47
W16	R	0.03	-0.41	0.30
W16	S	0.09	-0.17	0.39
W16	T	0.03	-0.31	0.41
W16	V	0.01	0.88	0.76
W16	W	1.00	1.00	1.00
W16	Y	0.22	1.09	1.02
V17	A	1.01	0.68	1.21
V17	E	0.82	0.75	1.11
V17	F	0.92	0.85	1.09
V17	G	1.17	0.84	0.93
V17	I	0.95	0.99	1.08
V17	K	0.94	0.84	1.06
V17	L	0.90	1.00	0.76
V17	P	0.77	0.96	0.97

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
V17	R	1.10	0.94	0.76
V17	S	0.96	1.04	0.89
V17	T	0.93	0.86	1.03
V17	V	1.00	1.00	1.00
V17	Y	0.91	0.88	0.99
P18	A	-0.28	-0.94	-0.03
P18	C	1.26	4.16	2.56
P18	E	1.22	4.87	2.47
P18	G	1.07	4.96	2.47
P18	H	1.12	6.05	2.50
P18	L	0.93	7.40	2.50
P18	N	1.33	1.42	2.35
P18	P	1.00	1.00	1.00
P18	Q	1.12	3.26	2.13
P18	R	1.16	3.97	2.01
P18	S	0.11	0.07	1.05
P18	V	1.19	4.85	2.30
P18	Y	1.33	4.17	1.68
V19	A	0.61	0.55	1.23
V19	D	0.77	0.79	0.80
V19	E	0.74	0.62	1.10
V19	G	1.32	0.56	1.39
V19	K	0.96	0.97	1.03
V19	L	1.00	0.91	0.90
V19	M	0.33	0.12	1.00
V19	P	0.00	-0.41	0.76
V19	Q	0.93	0.40	1.07
V19	R	1.03	0.34	0.82
V19	S	1.24	0.57	0.80
V19	V	1.00	1.00	1.00
V19	Y	0.94	0.70	0.92
E20	A	1.29	1.28	1.08
E20	C	1.57	1.76	0.71
E20	D	0.87	1.14	0.97
E20	E	1.00	1.00	1.00
E20	G	2.36	0.78	1.11

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
E20	H	2.17	1.20	0.92
E20	L	2.20	0.73	0.92
E20	N	1.40	1.34	1.01
E20	P	1.00	1.43	1.08
E20	Q	1.27	1.56	0.99
E20	S	2.01	1.18	0.91
E20	T	2.22	1.25	0.94
E20	V	2.11	1.27	1.01
E20	W	2.94	1.30	0.79
D21	A	1.46	1.75	0.84
D21	D	1.00	1.00	1.00
D21	E	0.84	1.39	0.85
D21	F	1.30	1.41	0.81
D21	G	1.37	1.76	0.93
D21	K	1.58	1.80	0.74
D21	L	1.46	1.57	0.82
D21	P	0.81	0.86	0.74
D21	S	1.24	1.11	0.73
D21	V	-0.17	-0.12	-0.02
D21	W	1.55	1.44	0.61
D21	Y	1.30	2.01	0.42
G22	A	1.55	1.66	1.07
G22	E	0.15	1.19	0.56
G22	G	1.00	1.00	1.00
G22	I	0.37	1.03	1.03
G22	K	0.23	-0.22	0.78
G22	L	0.38	1.35	0.84
G22	P	0.28	1.36	0.80
G22	Q	0.35	1.44	0.96
G22	R	0.11	0.56	0.73
G22	S	1.02	0.98	0.94
G22	T	1.03	1.16	0.80
G22	V	0.40	0.85	0.89
G22	W	0.25	0.23	0.58
A23	A	1.00	1.00	1.00
A23	F	0.05	0.44	1.03
A23	G	0.45	0.35	0.93
A23	H	0.16	1.04	0.93
A23	L	0.30	1.30	0.75
A23	M	0.85	0.95	0.90
A23	P	-0.11	0.73	0.82
A23	Q	0.23	0.73	0.91
A23	R	0.11	0.28	0.80
A23	S	0.69	0.34	0.87
A23	V	0.20	0.60	0.73
A23	W	0.29	0.80	0.71
A23	Y	0.20	0.96	0.73
P24	A	0.54	0.68	0.88
P24	C	0.54	1.04	0.87
P24	G	0.49	0.76	1.34
P24	H	0.42	0.97	1.15
P24	I	0.42	0.85	1.11
P24	K	0.52	1.36	0.71
P24	L	0.58	1.51	1.06
P24	P	1.00	1.00	1.00
P24	Q	0.50	0.65	0.93
P24	R	0.58	0.91	0.85
P24	S	0.53	0.61	1.31
P24	T	0.44	0.66	1.43
T25	A	1.33	0.86	1.23
T25	C	0.67	0.51	1.37
T25	D	0.03	-0.07	0.87
T25	E	0.08	-0.29	0.98
T25	G	1.86	0.43	1.27
T25	H	0.42	-0.02	0.94
T25	I	1.02	0.35	1.19
T25	K	0.36	0.13	0.87
T25	L	0.40	-0.04	0.95
T25	M	0.29	-0.10	1.04
T25	P	0.97	-0.05	1.10
T25	R	0.32	-0.06	0.94
T25	S	1.60	0.58	0.95

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Table 10-12, Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T25	T	1.00	1.00	1.00
T25	V	0.91	0.51	1.30
T25	W	0.33	0.14	0.86
E26	A	1.93	1.45	0.79
E26	C	1.40	0.94	0.82
E26	D	0.65	1.39	0.90
E26	E	1.00	1.00	1.00
E26	G	1.28	0.87	0.82
E26	H	1.33	1.19	0.71
E26	K	1.46	1.47	0.77
E26	L	1.30	1.71	0.77
E26	M	2.00	1.10	0.89
E26	N	1.37	0.48	0.88
E26	P	0.43	0.99	0.63
E26	R	1.48	0.81	0.77
E26	S	1.27	0.28	0.92
E26	T	1.44	0.40	0.82
E26	V	1.39	0.97	0.85
E26	W	1.25	0.47	0.68
R27	A	0.45	2.78	0.67
R27	C	0.35	0.58	0.50
R27	E	0.58	0.93	0.46
R27	G	0.42	0.84	0.24
R27	I	0.72	1.41	0.70
R27	K	1.22	1.55	0.69
R27	L	0.48	2.60	0.51
R27	P	0.93	0.48	0.46
R27	R	1.00	1.00	1.00
R27	S	0.53	0.69	0.56
R27	T	0.41	0.01	0.74
R27	V	0.71	0.94	0.85
R27	W	0.21	-0.59	0.33
F28	A	1.27	1.48	0.92
F28	C	0.93	1.21	0.87
F28	D	0.67	2.07	0.40
F28	E	0.51	1.04	0.85

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
F28	F	1.00	1.00	1.00
F28	G	0.74	-1.53	0.50
F28	H	-0.20	-0.19	-0.01
F28	I	-0.20	-0.19	-0.01
F28	L	1.09	2.02	0.51
F28	M	1.33	1.37	0.70
F28	P	0.02	0.39	0.42
F28	R	-0.20	-0.19	-0.01
F28	S	1.05	0.70	0.82
F28	V	0.86	0.53	0.85
F28	W	1.16	1.17	0.89
F28	Y	0.99	1.36	0.77
A29	A	1.00	1.00	1.00
A29	C	1.08	1.15	0.76
A29	D	0.87	1.00	1.06
A29	E	1.12	0.84	1.02
A29	G	1.60	0.80	1.22
A29	M	0.67	0.77	1.06
A29	P	0.78	0.62	1.07
A29	R	1.76	0.73	0.81
A29	S	1.49	0.55	1.05
A29	T	1.42	0.47	1.02
A29	V	1.80	0.44	1.05
A29	W	1.91	0.74	0.82
A29	Y	1.70	0.59	0.96
P30	A	1.05	0.92	1.15
P30	E	1.01	1.24	1.20
P30	G	0.90	1.09	0.99
P30	H	1.01	1.08	1.05
P30	I	0.97	1.38	0.95
P30	K	1.21	1.39	1.06
P30	L	0.96	1.17	1.07
P30	M	0.96	0.79	0.94
P30	P	1.00	1.00	1.00
P30	Q	1.01	0.91	1.06
P30	R	1.16	1.14	0.94

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P30	S	1.03	1.49	1.12
P30	T	1.05	1.64	1.00
P30	V	1.06	1.74	0.99
P30	Y	0.79	1.31	1.04
D31	A	1.24	1.18	0.80
D31	D	1.00	1.00	1.00
D31	E	1.13	0.88	0.93
D31	F	1.44	1.39	0.65
D31	G	1.44	1.16	0.79
D31	L	1.81	1.61	0.65
D31	N	1.34	1.55	0.62
D31	Q	1.07	1.13	0.74
D31	R	1.22	1.49	0.50
D31	S	1.15	1.23	0.55
D31	T	1.45	1.11	0.76
D31	V	1.28	1.08	0.50
D31	W	1.83	1.14	0.60
V32	A	0.43	3.64	1.10
V32	D	0.45	4.19	0.95
V32	E	0.57	3.92	1.00
V32	G	0.58	2.65	0.98
V32	I	0.91	3.51	1.08
V32	K	1.09	4.73	0.75
V32	L	0.96	4.72	1.01
V32	M	0.64	3.41	1.11
V32	N	0.54	1.61	0.99
V32	P	0.01	-1.17	0.31
V32	Q	0.64	1.74	1.03
V32	R	1.05	0.72	0.51
V32	S	0.77	1.09	0.85
V32	V	1.00	1.00	1.00
V32	W	0.94	1.71	0.70
R33	A	0.20	1.32	0.52
R33	C	0.44	1.73	0.95
R33	D	-0.16	-0.30	-0.02
R33	E	-0.16	-0.30	-0.02
R33	G	0.64	2.63	0.47
R33	H	-0.16	-0.30	-0.02
R33	K	0.85	2.72	0.81
R33	L	0.34	2.90	0.74
R33	N	0.90	1.30	0.92
R33	P	-0.16	-0.30	-0.02
R33	R	1.00	1.00	1.00
R33	S	1.00	1.01	0.79
R33	V	0.50	0.94	0.89
R33	W	-0.16	-0.30	-0.02
W34	A	-0.15	2.29	0.41
W34	C	-0.15	1.49	0.52
W34	E	-0.15	-1.86	0.17
W34	G	0.12	0.88	0.23
W34	I	0.18	0.94	0.75
W34	K	-0.15	-0.15	-0.02
W34	M	0.16	1.22	0.91
W34	P	-0.15	1.21	0.26
W34	Q	0.02	0.04	0.25
W34	R	0.22	-0.33	0.16
W34	S	0.47	0.08	0.29
W34	T	0.36	0.15	0.29
W34	V	0.24	0.73	0.71
W34	W	1.00	1.00	1.00
T35	A	0.45	3.85	0.98
T35	C	0.55	4.72	1.16
T35	E	0.30	5.73	0.26
T35	I	0.63	5.38	0.45
T35	K	-0.13	-0.54	-0.01
T35	L	-0.13	-0.54	-0.01
T35	M	0.17	2.72	0.40
T35	N	0.20	-2.29	0.43
T35	P	-0.13	-0.54	-0.01
T35	Q	0.57	-2.07	0.52
T35	R	0.18	-11.34	0.23
T35	T	1.00	1.00	1.00

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T35	V	0.71	0.34	0.81
T35	W	-0.13	-0.54	-0.01
T35	Y	-0.13	-0.54	-0.01
G36	A	0.63	1.07	1.00
G36	C	0.53	1.06	1.09
G36	D	-0.12	2.50	0.28
G36	G	-0.12	-0.10	-0.02
G36	H	0.73	1.10	0.98
G36	I	1.32	1.81	0.31
G36	K	1.27	1.71	0.84
G36	L	1.24	2.49	0.39
G36	M	0.85	0.54	0.85
G36	N	0.49	0.56	1.08
G36	P	-0.12	-0.10	-0.02
G36	Q	0.56	0.71	1.07
G36	R	0.99	0.90	0.85
G36	S	0.78	0.26	1.06
G36	T	0.76	0.33	0.83
G36	V	0.95	-0.38	0.42
G36	W	0.91	0.68	0.57
V37	A	1.25	2.00	0.63
V37	C	1.09	1.63	0.68
V37	H	1.21	0.96	0.78
V37	I	1.26	1.04	0.77
V37	L	1.16	1.16	0.71
V37	N	0.90	1.52	1.09
V37	P	0.53	2.10	0.73
V37	Q	-0.11	-0.14	-0.02
V37	R	-0.11	-0.14	-0.02
V37	S	1.40	1.49	0.81
V37	T	1.05	0.81	0.63
V37	V	-0.11239	-0.14412	-0.02
V37	W	0.92	0.98	0.62
L38	A	0.59	0.63	0.78

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L38	C	0.64	0.72	0.89
L38	D	-0.15	0.12	0.24
L38	E	-0.15	-0.61	0.26
L38	G	0.15	-0.72	0.32
L38	K	0.63	-0.22	0.16
L38	L	1.00	1.00	1.00
L38	P	-0.15	-0.78	0.28
L38	Q	-0.15	-0.02	0.47
L38	R	-0.15	-0.96	0.34
L38	S	0.38	0.29	0.48
L38	V	0.88	1.12	0.73
L38	W	-0.15	-0.11	-0.02
A39	A	1.00	1.00	1.00
A39	C	0.63	0.92	0.50
A39	E	1.09	0.83	1.03
A39	F	-0.17	-0.11	-0.02
A39	G	1.17	0.30	0.92
A39	I	1.26	0.71	0.91
A39	K	1.36	0.96	0.90
A39	L	1.43	0.97	0.93
A39	M	0.52	0.81	0.46
A39	N	0.51	0.43	0.45
A39	P	0.69	0.74	0.45
A39	R	1.17	0.64	0.94
A39	S	0.49	-4.31	0.16
A39	T	1.26	0.79	0.92
A39	V	1.21	0.98	1.18
A39	W	1.23	1.02	0.94
A39	Y	1.36	1.13	0.90
Q40	D	1.16	1.59	0.69
Q40	E	1.08	1.28	0.81
Q40	G	1.79	2.17	0.93
Q40	I	2.58	1.10	0.49
Q40	K	2.61	3.64	0.52
Q40	L	2.14	1.49	0.53
Q40	N	1.53	1.00	0.78

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Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
Q40	P	0.45	-0.19	0.24
Q40	Q	1.00	1.00	1.00
Q40	R	1.89	1.48	0.61
Q40	S	1.57	1.65	0.87
Q40	T	2.01	1.81	0.75
Q40	W	2.39	2.59	0.54
Q40	Y	1.83	2.02	0.65
Q41	A	1.03	2.58	0.73
Q41	G	0.97	1.09	0.77
Q41	H	1.12	1.14	0.89
Q41	K	1.38	1.61	0.70
Q41	L	1.00	1.92	0.79
Q41	P	0.21	0.66	0.45
Q41	Q	1.00	1.00	1.00
Q41	R	1.19	1.27	0.74
Q41	S	1.11	0.22	0.92
Q41	V	1.07	-0.05	0.90
Q41	W	1.14	0.88	0.71
Q41	Y	1.09	0.70	0.82
L42	C	0.76	1.43	0.68
L42	D	-0.14	-0.17	-0.02
L42	F	1.07	1.02	0.48
L42	G	1.17	0.76	0.50
L42	H	1.92	-0.33	0.15
L42	I	0.97	0.66	0.83
L42	K	2.46	1.41	0.13
L42	L	1.00	1.00	1.00
L42	M	0.78	0.74	0.95
L42	P	0.71	1.34	0.23
L42	Q	0.57	0.28	0.40
L42	R	1.38	0.64	0.15
L42	S	0.97	0.45	0.46
L42	T	1.08	-0.04	0.41
L42	V	0.91	0.73	0.74
L42	W	2.06	-0.70	0.14
G43	A	1.49	1.07	0.45

Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
G43	C	1.48	0.73	0.36
G43	E	1.25	1.88	0.66
G43	G	1.00	1.00	1.00
G43	H	1.17	0.96	0.63
G43	I	0.94	0.77	0.42
G43	K	1.42	0.86	0.65
G43	L	1.22	1.82	0.42
G43	M	1.37	0.88	0.28
G43	P	1.08	0.31	0.65
G43	Q	0.91	0.48	0.63
G43	R	1.22	0.59	0.57
G43	S	1.18	0.23	0.79
G43	V	0.93	0.33	0.44
G43	Y	1.26	0.94	0.36
A44	A	1.00	1.00	1.00
A44	C	1.80	1.92	0.46
A44	D	-0.17	-0.11	-0.01
A44	E	-0.17	0.03	0.10
A44	F	2.84	0.80	0.99
A44	H	-0.17	-0.11	-0.01
A44	L	1.61	0.99	0.87
A44	M	1.20	0.98	0.71
A44	P	-0.17	-0.11	-0.01
A44	R	0.29	-2.17	0.08
A44	S	0.52	-0.92	0.16
A44	T	0.30	-1.11	0.14
A44	V	2.13	0.50	0.94
A44	W	1.40	0.85	0.61
A44	Y	0.30	-0.23	0.10
D45	A	1.04	0.84	0.99
D45	C	0.83	0.84	0.48
D45	D	1.00	1.00	1.00
D45	F	1.11	1.04	0.66
D45	G	1.13	0.84	0.94
D45	H	1.13	0.78	0.70
D45	K	1.34	0.87	0.86

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
D45	L	1.05	0.78	0.55
D45	M	0.86	0.78	0.88
D45	P	0.75	0.53	0.72
D45	Q	1.04	0.57	0.81
D45	R	1.16	0.49	0.72
D45	S	1.13	0.38	0.95
D45	T	1.27	0.44	0.86
D45	V	1.05	0.50	0.70
D45	W	1.15	0.58	0.54
F46	A	0.92	1.25	1.05
F46	C	0.84	1.16	1.01
F46	D	1.17	1.39	0.54
F46	E	1.25	1.31	0.38
F46	F	1.00	1.00	1.00
F46	G	1.02	0.94	0.61
F46	H	-0.13	-0.13	-0.01
F46	I	0.90	0.88	0.91
F46	K	1.00	1.46	0.48
F46	L	0.78	1.54	0.74
F46	M	0.78	1.42	0.81
F46	P	0.64	1.50	0.26
F46	S	0.73	0.66	0.72
F46	T	0.86	0.43	0.79
F46	V	0.82	0.79	0.89
F46	W	0.94	0.63	0.91
E47	A	0.95	0.76	0.84
E47	C	0.83	0.77	0.99
E47	D	0.99	0.98	0.97
E47	E	1.00	1.00	1.00
E47	F	1.09	0.76	0.96
E47	G	1.20	1.10	0.76
E47	H	1.27	0.99	0.93
E47	I	1.03	1.15	1.02
E47	K	1.19	1.06	0.89
E47	L	1.00	1.02	0.96
E47	M	0.90	0.70	0.84

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
E47	N	0.91	0.63	0.99
E47	P	1.36	0.36	0.49
E47	R	2.45	0.62	0.75
E47	S	1.28	0.63	0.83
E47	T	1.96	0.84	0.98
V48	A	0.60	1.63	0.47
V48	C	0.83	2.25	0.91
V48	E	0.02	0.99	0.18
V48	F	0.67	1.42	0.57
V48	G	0.61	0.87	0.25
V48	L	0.92	2.29	0.91
V48	M	0.85	1.79	0.71
V48	N	-0.15	0.98	0.23
V48	P	0.21	3.08	0.34
V48	Q	0.19	1.39	0.32
V48	R	0.76	-1.17	0.15
V48	S	0.65	0.42	0.40
V48	V	1.00	1.00	1.00
V48	W	-0.15	-0.19	-0.02
I49	A	0.92	1.87	0.58
I49	E	1.02	0.88	0.75
I49	G	1.34	1.12	0.28
I49	H	1.27	0.74	0.77
I49	I	1.00	1.00	1.00
I49	K	1.23	1.26	0.72
I49	L	1.14	1.03	0.93
I49	M	1.01	1.02	0.69
I49	P	0.47	0.16	0.29
I49	R	1.05	0.29	0.56
I49	S	1.24	0.79	0.70
I49	V	1.20	0.97	0.94
I49	W	0.70	0.68	0.64
I49	Y	1.07	1.02	0.82
E50	A	1.12	1.23	0.58
E50	D	0.78	1.22	0.80
E50	E	1.00	1.00	1.00

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
E50	G	0.93	1.11	0.60
E50	I	0.84	0.58	0.67
E50	L	1.19	0.97	0.41
E50	M	1.18	1.04	0.38
E50	P	0.85	1.02	0.71
E50	Q	0.98	0.91	0.70
E50	R	0.46	-0.77	0.20
E50	S	0.87	0.65	0.76
E50	V	1.00	0.43	0.81
E50	W	0.75	0.14	0.19
E51	A	1.28	2.72	0.74
E51	D	0.66	1.28	0.91
E51	E	1.00	1.00	1.00
E51	G	1.22	1.34	0.84
E51	I	1.07	0.04	0.52
E51	K	0.38	2.00	0.36
E51	L	1.11	0.93	0.57
E51	M	0.40	1.20	0.84
E51	P	-0.12	-0.39	-0.02
E51	Q	0.98	0.76	0.84
E51	R	0.35	-0.97	0.29
E51	T	1.18	1.17	0.48
E51	V	1.47	0.37	0.70
E51	W	0.44	0.17	0.22
G52	A	0.54	0.79	0.90
G52	E	-0.12	0.55	0.41
G52	F	-0.12	-0.08	0.52
G52	G	1.00	1.00	1.00
G52	H	0.18	-0.60	0.49
G52	I	0.10	0.07	0.80
G52	L	0.17	0.24	0.58
G52	M	0.05	-0.64	0.56
G52	P	-0.12	0.24	0.76
G52	Q	-0.12	0.28	0.52
G52	R	-0.12	0.35	0.18
G52	S	0.13	-0.18	0.83

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
G52	T	0.10	-0.17	0.76
G52	V	0.10	-0.16	0.86
G52	W	0.92	2.47	0.13
L53	D	0.01	0.01	0.72
L53	E	0.88	0.19	0.77
L53	G	1.32	0.33	0.80
L53	H	5.05	1.70	0.27
L53	I	0.55	0.66	0.88
L53	K	0.89	0.24	0.70
L53	L	1.00	1.00	1.00
L53	P	-0.11	-0.64	0.07
L53	Q	1.48	0.72	0.89
L53	R	0.20	-0.02	0.66
L53	S	1.16	0.26	0.95
L53	T	1.02	0.84	0.75
L53	V	0.52	0.65	0.88
L53	W	0.02	-0.07	0.77
S54	A	3.46	1.41	1.33
S54	C	1.26	0.88	1.21
S54	D	-0.17	0.65	1.08
S54	E	-0.17	0.30	1.16
S54	F	0.74	-0.14	0.91
S54	G	1.43	0.17	0.93
S54	H	-0.17	0.00	1.06
S54	I	4.78	0.12	0.94
S54	K	1.44	0.08	0.78
S54	L	2.02	0.26	0.59
S54	M	0.01	0.48	1.01
S54	N	0.29	1.29	1.01
S54	P	5.20	1.30	0.98
S54	Q	1.03	0.53	0.99
S54	R	3.38	0.35	0.84
S54	S	1.00	1.00	1.00
S54	T	1.46	0.33	0.88
S54	V	4.72	0.29	0.95
S54	W	0.11	-0.07	0.83

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
S54	Y	0.37	0.12	0.89
A55	A	-0.11	-0.15	-0.01
A55	C	0.14	1.26	0.98
A55	G	1.69	0.73	0.98
A55	H	0.04	0.92	0.93
A55	I	0.34	-0.43	0.80
A55	K	0.52	1.08	0.68
A55	L	0.11	0.87	0.81
A55	N	0.34	1.05	1.12
A55	P	-0.11	-0.01	0.84
A55	R	0.56	0.25	0.99
A55	S	0.76	0.87	1.08
A55	T	1.69	0.42	0.91
A55	V	0.49	-0.51	0.96
A55	W	0.00	-0.05	0.88
A55	Y	0.00	0.18	0.94
R56	A	0.22	0.69	0.85
R56	C	0.45	-0.02	0.93
R56	E	-0.12	-0.04	0.16
R56	G	0.30	-0.59	0.56
R56	H	-0.12	-0.37	-0.02
R56	K	-0.12	-0.37	-0.02
R56	L	0.05	0.24	0.87
R56	N	0.18	0.27	0.31
R56	P	-0.12	-0.37	-0.02
R56	Q	0.01	-0.01	1.02
R56	R	1.00	1.00	1.00
R56	S	0.39	0.12	0.55
R56	T	0.10	-0.37	0.85
R56	W	-0.12	-0.37	-0.02
R56	Y	-0.12	-0.37	-0.02
T57	A	0.60	0.65	0.59
T57	C	0.60	0.40	0.85
T57	G	0.92	1.05	0.53
T57	H	0.83	0.61	0.23
T57	I	1.19	0.87	0.65
T57	L	0.63	0.76	0.95
T57	N	0.89	0.25	0.69
T57	P	0.33	-0.87	0.13
T57	R	1.61	-0.66	0.14
T57	S	1.63	1.01	0.88
T57	T	1.00	1.00	1.00
T57	V	1.28	0.87	0.84
T57	W	-0.08	-0.10	-0.01
T57	Y	0.52	0.55	0.43
T58	A	0.65	0.36	0.76
T58	E	-0.19	-0.10	-0.02
T58	G	-0.19	-0.10	-0.02
T58	H	0.89	1.49	0.74
T58	K	-0.19	-0.10	-0.02
T58	L	0.88	1.12	0.78
T58	M	0.56	0.03	0.50
T58	P	-0.19	-0.10	-0.02
T58	R	-0.19	-0.10	-0.02
T58	S	0.82	0.96	0.90
T58	T	1.00	1.00	1.00
T58	V	0.56	0.96	1.13
T58	W	-0.19	-0.10	-0.02
T58	Y	-0.19	-0.10	-0.02
N59	A	0.35	10.44	0.73
N59	C	0.40	11.23	0.78
N59	D	0.52	11.72	0.67
N59	E	0.66	9.88	0.38
N59	F	0.82	10.23	0.57
N59	G	0.88	10.00	0.66
N59	K	0.89	8.21	0.31
N59	L	0.88	14.74	0.32
N59	M	0.42	-1.42	0.72
N59	N	1.00	1.00	1.00
N59	P	0.12	-55.11	0.14
N59	Q	1.02	1.86	0.73
N59	R	1.09	-11.28	0.39

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
N59	S	1.06	7.32	0.74
N59	T	1.07	5.63	0.56
N59	V	0.81	9.97	0.96
N59	W	1.13	12.80	0.59
N59	Y	0.80	11.14	0.61
I60	A	0.81	0.79	1.20
I60	C	0.69	0.67	0.97
I60	D	0.83	0.66	0.56
I60	E	0.87	0.92	0.83
I60	G	1.00	1.04	0.86
I60	H	1.02	1.07	0.96
I60	I	1.00	1.00	1.00
I60	K	0.99	0.96	0.73
I60	L	0.95	0.91	1.02
I60	M	0.96	0.68	1.14
I60	P	0.23	0.32	0.31
I60	R	1.00	0.81	0.79
I60	S	0.78	1.00	0.92
I60	V	0.87	1.06	1.06
I60	Y	0.78	1.19	0.89
D61	A	0.70	0.71	1.41
D61	C	0.79	0.85	0.92
D61	D	1.00	1.00	1.00
D61	F	1.01	0.70	0.61
D61	G	0.81	1.25	0.84
D61	H	1.44	1.67	0.97
D61	I	1.08	1.66	0.98
D61	K	0.92	1.72	0.97
D61	L	0.80	1.20	1.00
D61	N	0.79	1.00	1.12
D61	P	0.83	1.13	0.97
D61	Q	0.89	1.16	1.02
D61	R	1.11	1.59	0.69
D61	S	1.26	1.35	0.97
D61	V	0.95	0.97	1.10
D61	Y	0.84	0.95	1.03

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
D62	A	-0.24	0.11	1.06
D62	C	0.52	0.49	0.96
D62	E	1.02	0.60	0.93
D62	G	0.28	-0.21	0.86
D62	H	0.61	-0.01	0.89
D62	I	0.72	-0.25	0.92
D62	L	0.51	-0.37	0.95
D62	M	0.03	-0.24	1.06
D62	P	-0.24	-0.55	0.69
D62	Q	-0.24	-0.35	0.86
D62	R	0.12	-0.81	0.62
D62	S	0.57	-0.10	0.88
D62	T	0.76	-0.41	0.76
D62	V	0.62	-0.26	0.87
D62	W	0.58	-0.45	0.79
P63	A	1.35	0.60	1.06
P63	F	1.25	0.93	0.97
P63	G	1.71	1.22	1.00
P63	K	1.40	1.02	0.99
P63	L	1.15	1.23	0.84
P63	M	1.46	0.91	1.09
P63	Q	1.09	1.05	1.08
P63	R	1.31	0.80	1.02
P63	S	1.42	0.90	1.17
P63	T	1.50	1.32	1.02
P63	V	1.31	1.04	1.06
P63	W	1.35	1.11	0.86
P63	Y	1.35	0.95	1.12
T64	A	0.96	1.20	0.97
T64	C	0.78	0.88	1.05
T64	D	0.87	0.64	0.81
T64	G	1.23	1.08	1.00
T64	H	0.89	0.96	0.90
T64	L	0.63	1.22	0.93
T64	M	0.68	1.09	1.07
T64	N	0.69	0.98	0.91

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T64	P	0.76	0.94	0.61
T64	Q	0.76	0.87	1.13
T64	R	0.15	0.11	1.05
T64	S	1.11	0.99	1.03
T64	T	1.00	1.00	1.00
T64	W	0.71	0.69	0.72
D65	A	1.31	0.72	0.72
D65	D	1.00	1.00	1.00
D65	G	0.80	0.52	0.88
D65	H	1.10	0.40	0.71
D65	I	0.53	0.62	0.46
D65	P	-0.33	0.42	0.08
D65	R	0.41	0.22	0.84
D65	S	1.17	0.47	0.76
D65	T	0.90	0.50	0.68
D65	V	0.88	0.20	0.64
D65	W	0.77	0.50	0.65
D65	Y	0.83	0.42	0.64
P66	A	0.50	0.56	1.03
P66	C	0.51	0.52	1.51
P66	D	1.00	0.72	0.90
P66	F	0.95	0.67	1.02
P66	G	1.50	0.44	1.78
P66	H	1.59	0.95	1.23
P66	I	1.59	0.84	1.51
P66	L	1.14	0.99	0.92
P66	N	1.12	0.38	1.62
P66	P	-0.09	-0.11	-0.01
P66	Q	1.46	0.42	1.91
P66	R	1.85	0.51	1.26
P66	S	1.39	1.02	0.98
P66	T	1.41	1.10	0.72
P66	V	1.83	0.89	1.12
P66	Y	1.33	0.70	1.08
R67	A	-0.20	0.22	1.39
R67	E	1.04	0.11	0.85

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
R67	F	1.26	0.01	1.01
R67	G	1.39	0.41	0.81
R67	K	0.91	0.99	0.76
R67	L	1.20	0.16	1.46
R67	N	1.58	0.33	1.00
R67	P	1.01	0.04	1.04
R67	Q	1.16	0.13	1.60
R67	R	1.00	1.00	1.00
R67	T	1.28	0.32	0.76
R67	V	0.89	0.12	1.24
R67	W	1.07	0.02	0.95
L68	A	0.59	-0.11	1.07
L68	C	0.76	0.06	0.85
L68	D	-0.16	0.44	0.55
L68	E	1.44	0.13	0.87
L68	F	0.70	0.25	1.00
L68	G	1.09	-0.08	1.00
L68	H	1.05	0.22	0.89
L68	I	1.13	0.73	0.86
L68	L	1.00	1.00	1.00
L68	M	0.59	0.03	0.99
L68	N	0.51	0.10	0.95
L68	P	0.29	0.35	0.82
L68	Q	0.50	0.25	0.90
L68	R	0.19	0.47	0.75
L68	S	0.99	0.07	0.93
L68	T	1.03	0.32	0.92
L68	V	1.09	0.51	1.01
L68	W	1.21	0.56	0.88
L68	Y	0.71	0.45	0.97
N69	A	0.92	1.13	0.93
N69	C	1.05	1.20	1.18
N69	D	0.90	1.11	1.05
N69	G	1.20	0.98	1.06
N69	H	1.36	1.52	0.73
N69	I	1.47	1.75	0.69

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
N69	K	1.72	1.59	0.84
N69	L	1.30	1.20	0.36
N69	N	1.00	1.00	1.00
N69	P	1.00	0.59	0.66
N69	Q	1.07	1.14	0.74
N69	R	1.49	0.83	0.84
N69	S	1.21	1.42	1.03
N69	T	1.35	1.43	0.87
N69	V	1.99	1.73	0.87
N69	W	1.05	0.55	0.36
N69	Y	0.88	0.17	0.44
G70	A	0.85	1.41	1.08
G70	C	0.12	-0.90	0.40
G70	E	-0.16	0.33	0.28
G70	F	0.00	-0.36	0.21
G70	G	1.00	1.00	1.00
G70	H	0.04	1.90	0.26
G70	I	0.04	0.27	0.33
G70	K	0.03	-0.80	0.26
G70	L	0.03	1.01	0.30
G70	M	0.62	-0.72	0.29
G70	N	0.02	-0.76	0.37
G70	P	0.16	-0.58	0.29
G70	Q	0.02	-0.83	0.36
G70	R	0.08	-1.84	0.25
G70	S	0.69	0.64	0.88
G70	T	0.27	-0.10	0.45
G70	V	0.16	-0.52	0.34
G70	Y	0.08	-0.33	0.38
A71	A	1.00	1.00	1.00
A71	C	1.01	0.99	0.85
A71	D	0.70	0.65	0.68
A71	E	1.45	0.81	0.83
A71	F	1.13	0.99	0.75
A71	G	1.59	0.68	0.85
A71	H	1.70	0.78	0.75

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
A71	I	1.51	0.79	0.81
A71	K	1.44	1.01	0.76
A71	L	1.23	0.84	0.85
A71	M	0.98	1.11	0.81
A71	N	1.23	0.61	0.77
A71	P	-0.14	-0.05	0.46
A71	R	1.40	0.77	0.71
A71	S	1.75	0.69	0.84
A71	T	1.70	0.79	0.83
S72	A	0.55	3.52	1.06
S72	C	0.56	2.18	0.96
S72	D	0.40	0.80	0.90
S72	E	0.61	0.93	0.99
S72	F	0.94	1.15	0.80
S72	G	1.20	1.76	0.87
S72	H	1.21	2.48	0.82
S72	L	1.26	0.70	1.07
S72	M	0.36	2.13	0.94
S72	N	0.42	2.85	0.99
S72	P	-0.25	0.56	0.63
S72	Q	0.62	0.66	0.98
S72	R	0.86	0.74	0.87
S72	S	1.00	1.00	1.00
S72	T	1.10	0.97	0.88
S72	V	1.08	0.83	0.90
S72	W	0.98	0.34	0.92
S72	Y	1.07	0.07	1.03
Y73	A	-0.10	1.40	0.82
Y73	C	-0.10	1.20	1.18
Y73	D	0.13	0.80	1.09
Y73	G	0.71	0.51	0.95
Y73	H	0.67	0.52	0.96
Y73	I	0.82	0.64	0.97
Y73	K	1.07	0.94	0.95
Y73	L	0.98	0.50	1.03
Y73	M	-0.10	1.13	1.05

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
Y73	N	0.56	0.76	1.25
Y73	P	0.64	-0.54	0.42
Y73	O	1.23	0.87	1.20
Y73	R	1.26	0.26	0.96
Y73	S	1.17	0.68	0.77
Y73	V	0.88	0.74	1.08
Y73	Y	-0.10	-0.10	-0.02
L74	A	0.07	2.90	1.01
L74	D	-0.18	-0.18	-0.03
L74	F	0.99	1.13	0.58
L74	G	1.95	0.57	0.18
L74	H	-0.18	-0.18	-0.03
L74	I	0.86	0.64	1.45
L74	L	1.00	1.00	1.00
L74	M	0.15	1.21	0.79
L74	P	-0.18	-0.18	-0.03
L74	Q	-0.18	-0.18	-0.03
L74	R	-0.18	-0.18	-0.03
L74	S	2.72	-1.52	0.25
L74	T	-0.18	-0.18	-0.03
L74	V	0.90	0.61	1.18
L74	W	1.38	0.67	0.50
L74	Y	0.90	0.86	1.19
P75	C	0.54	1.42	1.06
P75	D	0.67	2.09	0.86
P75	E	0.83	1.19	1.00
P75	G	1.16	0.93	0.81
P75	H	1.05	0.86	0.89
P75	I	0.69	0.74	0.78
P75	K	0.60	0.88	0.91
P75	L	0.44	1.19	1.02
P75	M	0.36	0.30	1.22
P75	P	1.00	1.00	1.00
P75	Q	1.21	0.61	1.04
P75	R	1.60	0.46	0.89
P75	S	1.39	0.63	1.18

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P75	T	1.28	0.69	1.10
P75	V	0.93	1.39	0.90
P75	W	1.04	1.31	0.84
P75	Y	0.69	1.32	1.08
S76	A	0.38	1.11	0.60
S76	C	0.39	1.06	0.67
S76	D	0.41	1.94	0.49
S76	E	0.47	2.09	0.58
S76	F	0.44	0.46	0.68
S76	G	0.64	2.15	0.69
S76	H	0.85	1.11	0.79
S76	K	0.59	1.53	0.32
S76	L	0.74	4.70	0.27
S76	M	0.49	1.61	0.45
S76	P	1.23	1.20	0.67
S76	O	0.84	0.90	0.88
S76	S	1.00	1.00	1.00
S76	T	0.75	1.11	0.80
S76	V	0.67	1.35	0.78
S76	W	0.57	-0.25	1.06
S76	Y	0.31	0.18	0.75
C77	A	0.83	0.91	1.20
C77	C	1.00	1.00	1.00
C77	D	0.92	1.05	0.45
C77	F	0.25	-0.61	0.75
C77	G	1.01	0.18	0.53
C77	L	0.98	0.73	1.44
C77	N	-0.13	-0.06	-0.04
C77	P	-0.13	-0.06	-0.04
C77	R	0.70	-1.02	0.34
C77	S	0.95	0.76	1.19
C77	T	1.12	1.03	1.18
C77	V	1.05	0.80	1.33
C77	W	0.39	-0.24	0.73
C77	Y	0.95	-0.01	0.66
L78	A	-0.11	-0.14	-0.01

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L78	C	0.92	0.78	0.91
L78	E	3.01	-1.14	0.16
L78	G	4.98	1.38	0.12
L78	H	4.82	1.57	0.25
L78	I	1.43	1.11	1.06
L78	L	1.00	1.00	1.00
L78	M	0.52	0.48	0.75
L78	N	2.68	-0.41	0.22
L78	P	-0.11	-0.14	-0.01
L78	Q	1.73	0.52	0.46
L78	R	-0.11	-0.14	-0.01
L78	S	-0.11	-0.14	-0.01
L78	T	1.87	1.10	0.47
L78	V	1.53	0.83	1.04
L78	Y	1.39	0.81	0.46
A79	A	-0.15	-0.13	-0.02
A79	C	0.97	0.03	1.16
A79	E	1.12	0.27	1.12
A79	F	-0.15	-2.02	0.17
A79	G	0.92	0.92	0.99
A79	H	1.93	-0.09	0.85
A79	I	1.59	0.67	0.87
A79	L	1.80	0.96	0.88
A79	M	1.50	0.28	1.04
A79	N	1.48	0.28	0.97
A79	P	0.70	0.94	0.81
A79	Q	1.47	0.27	1.05
A79	R	1.47	0.32	1.02
A79	S	0.82	0.78	1.09
A79	T	1.17	0.60	0.90
A79	V	-0.15	-0.13	-0.02
A79	W	1.27	0.53	0.46
T80	A	1.00	1.11	0.90
T80	C	1.31	1.15	0.91
T80	E	0.07	-0.16	1.02
T80	G	1.16	1.50	0.81

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T80	H	0.21	0.05	0.66
T80	I	0.50	0.15	0.78
T80	K	0.15	-0.32	0.74
T80	L	0.15	-0.11	0.68
T80	N	0.53	0.53	0.97
T80	P	-0.11	-0.05	0.55
T80	Q	0.91	1.07	1.02
T80	R	0.08	-0.22	0.78
T80	S	0.96	1.40	1.12
T80	T	1.00	1.00	1.00
T80	V	1.23	1.01	0.93
T80	W	0.23	-0.86	0.46
T80	Y	0.15	0.11	0.69
H81	A	1.15	1.45	0.98
H81	C	1.13	1.09	0.92
H81	F	1.10	0.90	0.87
H81	G	1.17	0.80	0.94
H81	H	1.00	1.00	1.00
H81	K	1.52	0.56	0.31
H81	L	1.23	1.03	0.93
H81	M	0.94	1.54	0.82
H81	N	1.17	1.00	0.82
H81	P	-0.10	0.72	0.42
H81	Q	0.85	0.75	1.00
H81	R	0.34	-0.29	0.85
H81	S	1.04	0.69	0.94
H81	V	1.10	0.71	0.89
H81	W	1.13	1.09	0.90
H81	Y	0.77	0.14	0.76
L82	A	0.62	0.98	1.00
L82	G	1.38	0.31	1.24
L82	H	1.33	0.47	0.95
L82	I	1.17	0.51	0.58
L82	K	1.19	0.51	1.03
L82	L	1.00	1.00	1.00
L82	M	0.65	1.06	1.07

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L82	P	1.46	0.52	1.11
L82	R	1.34	-0.18	1.15
L82	S	1.15	0.00	1.13
L82	T	1.18	0.38	0.97
L82	V	1.02	0.19	1.14
L82	W	0.27	-0.46	0.93
P83	A	0.36	2.36	0.66
P83	C	0.53	1.01	0.81
P83	D	0.75	0.83	0.92
P83	E	0.84	1.26	0.92
P83	F	0.76	0.99	0.69
P83	G	1.31	0.68	1.01
P83	H	1.27	0.61	0.93
P83	K	1.37	1.16	0.88
P83	L	0.04	0.21	0.19
P83	M	0.58	1.88	0.71
P83	N	0.70	1.10	0.90
P83	P	1.00	1.00	1.00
P83	Q	0.73	0.82	0.95
P83	R	1.19	1.09	0.78
P83	S	1.17	0.79	0.89
P83	T	0.86	-0.02	0.62
P83	V	0.78	0.19	0.72
P83	W	0.98	0.62	0.69
L84	A	0.45	0.45	0.76
L84	D	0.19	0.85	0.48
L84	F	0.72	1.01	0.74
L84	G	0.77	1.01	0.53
L84	H	1.01	0.99	0.66
L84	I	0.90	0.87	0.99
L84	K	1.10	0.79	0.59
L84	L	1.00	1.00	1.00
L84	N	0.54	0.67	0.86
L84	P	-0.12	0.43	0.58
L84	Q	0.41	0.52	0.93
L84	R	0.56	0.57	0.71
L84	S	0.75	0.55	0.93
L84	T	0.86	0.44	0.95
L84	V	0.79	0.42	1.23
L84	W	0.36	-0.28	0.91
D85	A	0.79	1.09	0.63
D85	C	0.88	1.50	0.56
D85	D	1.00	1.00	1.00
D85	E	1.12	1.25	0.97
D85	F	1.01	1.98	0.52
D85	G	1.41	1.60	0.69
D85	H	1.55	1.24	0.76
D85	I	0.55	0.10	0.46
D85	L	0.53	0.24	0.52
D85	N	1.54	0.78	0.86
D85	P	0.97	0.54	0.63
D85	Q	3.09	0.99	0.82
D85	R	2.38	1.03	0.66
D85	S	2.28	0.68	0.93
D85	T	1.33	0.71	0.77
D85	V	0.61	0.25	0.65
D85	W	0.87	0.34	0.72
D85	Y	0.98	0.55	0.78
L86	A	1.38	3.32	0.40
L86	C	1.16	2.44	0.85
L86	E	0.06	-0.92	0.46
L86	F	-0.15	-0.26	-0.02
L86	G	1.15	0.70	0.83
L86	H	0.88	-0.72	0.57
L86	L	1.00	1.00	1.00
L86	P	-0.15	0.99	0.22
L86	Q	-0.15	-2.60	3.66
L86	R	0.43	-4.46	0.26
L86	S	0.78	-0.36	0.78
L86	T	0.96	0.28	0.75
L86	V	0.92	0.12	0.93
L86	W	0.67	0.08	0.78

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Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L86	Y	0.85	0.82	0.92
V87	A	0.65	0.17	0.88
V87	C	0.67	2.22	0.93
V87	D	-0.09	-2.53	0.32
V87	F	0.60	0.10	0.56
V87	G	0.46	-2.95	0.54
V87	K	0.04	-8.34	0.26
V87	L	0.71	4.30	0.84
V87	M	0.73	0.75	0.86
V87	P	0.07	1.64	0.39
V87	R	0.07	-1.33	0.44
V87	S	0.59	-0.09	0.67
V87	T	0.63	0.15	0.71
V87	V	1.00	1.00	1.00
V87	Y	0.33	-1.24	0.42
I88	G	1.01	-2.63	0.27
I88	H	1.20	-6.25	0.21
I88	I	1.00	1.00	1.00
I88	M	0.24	1.09	0.86
I88	N	-0.14	-0.55	0.29
I88	P	-0.14	3.51	0.18
I88	Q	0.01	-1.10	0.36
I88	R	-0.14	-0.32	-0.02
I88	T	1.03	-0.16	0.52
I88	Y	-0.14	-0.32	-0.02
I89	A	0.55	1.83	0.63
I89	D	-0.10	-0.14	-0.02
I89	E	-0.10	-2.05	0.24
I89	F	0.68	0.75	0.90
I89	G	0.64	-3.84	0.29
I89	H	1.00	-1.01	0.33
I89	I	1.00	1.00	1.00
I89	L	0.87	1.22	1.07
I89	P	0.38	1.91	0.30
I89	Q	0.25	-0.30	0.32
I89	R	-0.10	-0.14	-0.02

Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
I89	S	0.71	-1.66	0.49
I89	T	0.94	0.90	0.60
I89	V	0.91	0.82	1.09
I89	W	0.53	-2.63	0.27
M90	A	0.78	1.41	0.67
M90	C	0.79	1.09	0.83
M90	D	-0.24	2.88	0.15
M90	E	-0.24	1.15	0.29
M90	G	0.57	-1.22	0.33
M90	I	1.13	0.66	0.74
M90	L	1.02	0.98	0.84
M90	M	1.00	1.00	1.00
M90	P	-0.24	-0.36	0.28
M90	Q	0.68	0.77	0.71
M90	R	-0.24	0.36	0.23
M90	S	1.06	-0.17	0.56
M90	T	1.27	0.15	0.59
M90	V	1.08	0.08	0.62
M90	W	0.79	-4.04	0.21
L91	A	0.57	1.45	0.81
L91	C	0.67	1.27	0.87
L91	D	-0.12	1.47	0.12
L91	E	-0.12	-0.51	0.13
L91	G	1.21	-0.58	0.17
L91	H	-0.12	-0.13	-0.01
L91	I	0.98	1.05	0.89
L91	K	-0.12	-0.13	-0.01
L91	L	1.00	1.00	1.00
L91	M	0.28	0.88	0.80
L91	P	-0.12	-0.13	-0.01
L91	Q	0.05	-0.14	0.18
L91	R	-0.12	-0.13	-0.01
L91	S	0.92	0.43	0.24
L91	T	1.06	-0.11	0.36
L91	V	0.94	0.79	0.72
L91	W	-0.12	-0.13	-0.01

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L91	Y	-0.12	-0.13	-0.01
G92	A	-0.10	-0.18	-0.02
G92	C	-0.10	2.05	0.18
G92	D	-0.10	-0.18	-0.02
G92	E	-0.10	-2.31	0.21
G92	F	-0.10	-3.24	0.17
G92	G	1.00	1.00	1.00
G92	L	-0.10	-0.18	-0.02
G92	M	-0.10	-0.18	-0.02
G92	P	-0.10	-0.18	-0.02
G92	R	-0.10	-0.18	-0.02
G92	S	1.26	-2.96	0.21
G92	T	-0.10	-0.18	-0.02
G92	V	1.49	-3.03	0.20
G92	W	-0.10	-0.18	-0.02
G92	Y	-0.10	-0.18	-0.02
T93	A	1.38	1.05	0.50
T93	C	1.08	0.95	0.64
T93	D	-0.18	0.23	0.22
T93	F	3.52	0.54	0.63
T93	P	-0.18	-0.19	-0.02
T93	Q	-0.18	-6.75	2.03
T93	R	-0.18	-0.19	-0.02
T93	S	0.89	0.49	0.89
T93	T	1.00	1.00	1.00
T93	V	-0.18	-0.19	-0.02
T93	W	-0.18	-0.19	-0.02
T93	Y	5.26	0.03	0.77
N94	A	-0.45	0.74	0.96
N94	C	0.01	0.07	0.94
N94	G	0.15	0.53	0.76
N94	H	0.11	-0.94	0.77
N94	L	0.61	-0.18	0.49
N94	M	-0.45	0.03	0.94
N94	N	1.00	1.00	1.00
N94	P	-0.45	0.79	0.40
N94	R	0.10	-8.20	0.19
N94	S	0.10	0.88	0.84
N94	T	0.25	-1.43	0.66
N94	V	0.15	-0.39	0.65
N94	W	0.10	-1.20	0.69
N94	Y	0.08	0.12	0.76
D95	A	-0.14	-0.14	-0.01
D95	C	-0.14	-0.14	-0.01
D95	D	1.00	1.00	1.00
D95	E	2.04	0.75	0.66
D95	G	-0.14	-0.14	-0.01
D95	H	-0.14	-0.14	-0.01
D95	K	-0.14	-0.14	-0.01
D95	L	-0.14	-0.14	-0.01
D95	N	-0.14	-0.14	-0.01
D95	Q	-0.14	-0.14	-0.01
D95	R	-0.14	-0.14	-0.01
D95	S	-0.14	-0.14	-0.01
D95	T	-0.14	-0.14	-0.01
D95	V	-0.14	-0.14	-0.01
D95	W	-0.14	-0.14	-0.01
D95	Y	-0.14	-0.14	-0.01
T96	A	0.36	4.20	1.32
T96	C	0.44	3.76	0.79
T96	F	0.53	1.24	0.69
T96	G	0.78	1.28	1.03
T96	I	0.95	-0.22	0.88
T96	L	0.92	1.93	0.93
T96	M	0.39	2.53	0.80
T96	P	-0.11	0.89	0.35
T96	R	0.17	0.14	0.50
T96	S	1.04	0.79	1.05
T96	T	1.00	1.00	1.00
T96	V	0.81	0.59	1.12
T96	W	0.38	-4.29	0.51
T96	Y	0.38	-3.73	0.59

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
K97	A	0.01	0.23	1.11
K97	D	-0.23	-0.17	-0.01
K97	G	0.84	-0.64	0.39
K97	I	0.74	-0.55	0.47
K97	K	1.00	1.00	1.00
K97	L	0.38	-0.28	0.30
K97	M	0.02	0.22	0.95
K97	P	0.16	0.27	0.36
K97	Q	1.14	0.00	0.73
K97	R	2.80	0.59	1.02
K97	S	0.28	-0.46	0.58
K97	T	0.22	-0.42	0.51
K97	V	0.31	-0.45	0.51
K97	W	0.42	-2.32	0.13
K97	Y	0.29	-0.65	0.38
A98	A	1.00	1.00	1.00
A98	C	1.30	1.42	1.00
A98	D	1.11	2.19	0.81
A98	G	1.57	0.56	0.97
A98	H	2.09	0.92	0.82
A98	I	2.05	0.65	0.72
A98	L	2.22	1.47	0.71
A98	N	1.24	1.40	1.01
A98	P	1.10	1.26	0.90
A98	S	1.73	0.65	1.17
A98	T	1.72	0.27	1.03
A98	Y	2.02	1.15	0.87
Y99	A	0.66	0.82	1.29
Y99	G	0.83	0.70	1.23
Y99	H	0.77	0.59	1.30
Y99	I	0.81	0.61	1.11
Y99	L	0.66	0.86	1.39
Y99	P	0.89	0.81	1.00
Y99	R	0.61	0.29	0.97
Y99	S	0.72	0.37	1.45
Y99	V	0.61	0.31	1.28
Y99	W	0.68	0.57	1.20
Y99	Y	1.00	1.00	1.00
F100	A	0.78	2.02	0.93
F100	C	0.73	1.28	0.78
F100	D	0.38	-0.03	0.33
F100	E	1.01	0.15	0.83
F100	F	1.00	1.00	1.00
F100	K	0.65	-0.60	0.53
F100	M	0.79	2.19	1.20
F100	N	0.91	1.45	1.12
F100	S	0.87	0.85	1.02
F100	T	0.95	1.42	0.71
F100	W	1.08	-0.03	1.06
R101	C	0.71	0.95	0.96
R101	D	0.85	0.80	1.02
R101	F	0.84	0.97	0.66
R101	I	0.79	0.96	0.68
R101	K	1.24	0.07	0.90
R101	L	0.83	1.12	1.33
R101	N	0.72	0.92	1.11
R101	P	0.50	0.86	0.75
R101	Q	0.86	0.11	1.03
R101	R	1.00	1.00	1.00
R101	V	0.74	0.44	0.90
R101	W	0.95	0.00	0.89
R101	Y	0.74	0.80	0.67
R102	A	0.19	1.79	0.98
R102	C	0.22	0.36	0.78
R102	D	0.01	0.68	0.26
R102	F	0.46	0.23	0.31
R102	G	0.44	0.27	0.43
R102	L	0.33	1.64	0.95
R102	P	-0.07	0.89	0.26
R102	Q	0.67	1.19	1.09
R102	R	1.00	1.00	1.00
R102	S	0.46	0.96	0.98

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
R102	V	0.28	0.61	0.80
R102	W	0.29	-1.03	0.34
R102	Y	0.40	1.29	0.70
T103	A	0.97	-9.64	0.89
T103	C	0.90	-6.91	0.89
T103	F	0.74	-3.39	0.85
T103	G	1.11	-5.27	1.20
T103	H	0.99	-4.15	1.14
T103	I	1.08	-5.15	0.89
T103	K	1.09	-4.36	1.05
T103	L	1.05	-1.86	0.88
T103	N	0.77	-6.03	1.07
T103	P	0.69	-5.11	1.01
T103	R	0.87	-6.30	0.96
T103	S	0.92	-1.36	1.14
T103	T	1.00	1.00	1.00
T103	V	0.95	-1.95	0.90
T103	W	1.26	-2.60	0.77
T103	Y	1.19	-4.68	0.88
P104	A	-0.41	-0.19	-0.04
P104	C	1.95	1.83	1.34
P104	E	1.84	1.97	1.37
P104	F	1.79	0.86	0.67
P104	G	2.67	0.98	1.25
P104	H	2.84	1.03	1.11
P104	I	2.43	2.05	1.07
P104	L	-0.41	-0.19	-0.04
P104	M	1.09	2.24	1.01
P104	N	1.62	1.44	1.32
P104	P	1.00	1.00	1.00
P104	Q	1.34	0.85	1.24
P104	R	1.62	-0.39	0.83
P104	S	2.48	0.53	1.44
P104	T	2.70	0.33	1.29
P104	V	2.59	1.02	1.40
P104	W	2.05	0.23	0.59

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L105	A	-0.11	-0.18	-0.02
L105	C	1.56	1.92	1.05
L105	E	-0.11	0.53	0.26
L105	F	1.30	1.73	0.95
L105	G	1.08	1.40	1.07
L105	H	0.85	1.23	1.07
L105	L	1.00	1.00	1.00
L105	M	-0.11	-0.18	-0.02
L105	P	1.71	0.90	1.00
L105	Q	0.94	1.04	1.03
L105	R	0.99	1.25	0.94
L105	S	0.93	0.61	0.95
L105	T	0.92	0.64	1.00
L105	V	0.15	-0.97	0.37
L105	W	1.28	1.71	0.78
L105	Y	0.72	0.62	1.18
D106	A	0.72	1.13	0.69
D106	C	1.01	1.10	0.80
D106	D	1.00	1.00	1.00
D106	E	1.08	1.09	1.02
D106	F	1.02	1.45	0.34
D106	G	1.18	1.45	0.67
D106	H	1.09	1.18	0.66
D106	I	1.04	0.92	0.45
D106	K	1.28	1.24	0.68
D106	L	1.20	1.00	0.56
D106	M	0.73	0.86	0.77
D106	N	0.92	0.64	0.91
D106	P	-0.17	0.63	0.18
D106	Q	0.92	0.62	0.94
D106	R	0.98	0.56	0.91
D106	S	0.98	1.02	0.81
D106	T	1.06	1.38	0.64
D106	V	0.98	1.68	0.61
D106	W	0.78	1.07	0.34
I107	A	0.81	0.80	0.83

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
I107	C	0.95	1.41	1.00
I107	E	2.55	-0.28	0.21
I107	F	0.99	-0.02	0.19
I107	G	1.76	-10.12	0.25
I107	H	-0.07	-0.20	-0.02
I107	I	1.00	1.00	1.00
I107	L	0.96	1.04	0.52
I107	N	1.81	0.93	0.56
I107	P	0.65	0.32	0.40
I107	Q	0.53	-0.02	0.43
I107	R	0.08	-2.75	0.28
I107	S	2.04	1.33	1.05
I107	T	0.64	1.53	0.95
I107	V	1.00	0.97	1.04
I107	W	-0.07	-0.20	-0.02
I107	Y	0.49	0.52	0.23
A108	A	-0.12	-0.07	-0.02
A108	D	-0.12	-0.07	-0.02
A108	E	0.14	0.61	0.25
A108	F	-0.12	-0.07	-0.02
A108	G	0.99	1.13	1.15
A108	H	-0.12	-0.07	-0.02
A108	I	-0.12	-0.07	-0.02
A108	K	0.60	2.97	0.31
A108	L	1.41	2.56	0.20
A108	N	-0.12	-0.07	-0.02
A108	P	-0.12	-0.07	-0.02
A108	Q	0.58	0.73	0.98
A108	R	-0.12	-0.07	-0.02
A108	S	0.94	1.00	1.14
A108	T	1.05	0.87	1.08
A108	V	0.76	0.95	0.99
L109	A	0.34	0.32	1.07
L109	D	1.00	0.11	1.15
L109	E	0.74	0.19	1.24
L109	F	0.83	0.32	1.11

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L109	G	0.82	0.51	0.88
L109	H	0.85	0.22	1.06
L109	I	1.05	0.14	1.21
L109	L	1.00	1.00	1.00
L109	M	0.74	0.63	1.00
L109	N	1.52	0.66	1.13
L109	P	0.79	0.43	0.35
L109	Q	1.18	0.22	1.08
L109	R	0.48	0.21	0.95
L109	S	0.79	0.38	0.94
L109	T	0.63	0.79	0.87
L109	V	0.52	0.54	1.06
L109	W	1.30	-0.02	0.88
L109	Y	1.16	0.83	0.79
G110	A	0.91	1.01	0.88
G110	C	0.35	1.43	0.56
G110	D	0.76	1.40	0.87
G110	E	0.26	1.76	0.46
G110	F	0.04	2.29	0.30
G110	G	1.00	1.00	1.00
G110	H	0.63	0.73	0.46
G110	I	0.06	0.23	0.32
G110	L	-0.20	-0.12	-0.02
G110	M	0.16	0.82	0.34
G110	N	0.70	0.77	0.89
G110	P	0.02	0.22	0.50
G110	Q	0.44	0.34	0.77
G110	R	0.05	0.48	0.45
G110	S	0.79	0.30	1.01
G110	T	0.45	-0.05	0.42
G110	W	-0.20	-1.18	0.20
G110	Y	0.01	-0.88	0.40
M111	A	0.65	1.02	0.89
M111	C	0.92	1.01	0.95
M111	D	-0.27	0.79	0.37
M111	E	0.25	0.67	0.56

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
M111	F	1.47	0.78	0.75
M111	G	0.85	0.32	0.44
M111	H	0.98	0.19	0.40
M111	I	1.95	1.03	0.91
M111	K	1.98	0.71	0.58
M111	L	1.55	0.67	0.93
M111	M	1.00	1.00	1.00
M111	N	0.49	1.31	0.79
M111	P	-0.27	0.57	0.39
M111	R	0.27	-0.99	0.34
M111	S	1.03	0.14	0.52
M111	T	1.49	0.76	0.77
M111	V	1.47	0.93	0.88
M111	W	0.96	1.23	0.30
M111	Y	1.43	1.06	0.65
S112	A	0.58	0.94	0.98
S112	E	0.71	1.16	1.05
S112	F	0.37	0.88	0.61
S112	H	1.00	0.38	0.93
S112	K	0.84	0.68	0.92
S112	L	1.03	1.00	0.80
S112	M	0.43	0.56	0.98
S112	N	0.52	0.85	1.09
S112	P	-0.19	-0.82	0.33
S112	R	0.20	-0.44	0.99
S112	S	1.00	1.00	1.00
S112	T	0.95	0.72	0.87
S112	V	0.86	0.48	0.73
S112	W	0.74	0.58	0.85
S112	Y	0.68	-0.10	0.90
V113	A	0.71	1.31	0.70
V113	C	0.87	0.94	1.06
V113	D	0.78	0.87	0.97
V113	E	0.91	0.94	0.99
V113	F	1.05	0.96	0.80
V113	G	0.96	0.58	0.89

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
V113	H	1.34	0.76	0.84
V113	K	1.19	0.72	0.92
V113	L	1.50	0.85	0.85
V113	M	0.78	1.06	0.93
V113	N	0.88	1.22	1.01
V113	P	0.72	1.14	0.65
V113	Q	1.03	1.11	0.94
V113	R	1.13	1.11	0.82
V113	S	0.80	0.57	0.91
V113	T	0.94	0.86	0.89
V113	V	1.00	1.00	1.00
V113	W	0.91	0.80	0.76
V113	Y	1.11	0.98	0.85
L114	A	0.78	1.07	1.03
L114	C	0.78	1.14	1.10
L114	E	0.32	-0.14	0.42
L114	F	-0.11	-0.21	-0.02
L114	G	0.96	1.14	0.78
L114	H	0.92	-0.55	0.21
L114	I	0.97	1.17	0.86
L114	K	-0.11	-0.21	-0.02
L114	L	1.00	1.00	1.00
L114	M	0.73	1.28	1.00
L114	N	0.65	0.77	0.95
L114	P	0.30	0.28	0.42
L114	Q	0.59	0.12	0.68
L114	R	-0.11	-0.21	-0.02
L114	S	0.87	0.55	0.72
L114	T	0.88	1.05	0.82
L114	V	0.91	0.60	0.84
L114	W	-0.11	-0.21	-0.02
L114	Y	-0.11	-0.21	-0.02
V115	A	0.60	1.19	1.11
V115	C	0.73	1.08	1.14
V115	D	-0.15	2.21	0.19
V115	F	0.54	1.69	0.32

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
V115	G	1.09	1.76	0.43
V115	H	-0.15	-0.13	-0.02
V115	I	1.05	0.99	1.14
V115	K	-0.15	-0.13	-0.02
V115	L	1.12	1.30	1.02
V115	M	0.48	1.32	1.05
V115	P	-0.15	2.21	0.26
V115	Q	-0.15	1.15	0.32
V115	R	0.10	1.63	0.21
V115	S	0.95	1.14	0.72
V115	T	1.15	1.28	0.72
V115	V	1.00	1.00	1.00
V115	W	1.23	2.48	0.17
V115	Y	1.03	2.07	0.28
T116	A	1.01	0.95	1.08
T116	C	0.89	1.05	1.30
T116	E	0.86	0.91	1.29
T116	G	1.10	0.90	1.44
T116	H	1.00	1.08	1.48
T116	I	0.80	0.76	0.82
T116	L	0.77	0.68	1.03
T116	M	0.83	1.39	1.28
T116	N	0.93	1.05	1.68
T116	P	0.74	0.84	0.99
T116	Q	0.95	0.77	1.29
T116	R	0.64	0.62	1.03
T116	S	0.88	0.96	1.24
T116	T	1.00	1.00	1.00
T116	V	0.86	0.57	0.85
T116	W	0.89	0.75	0.96
T116	Y	0.90	0.47	1.09
Q117	A	2.05	1.73	1.03
Q117	E	1.15	1.21	1.10
Q117	F	1.57	1.02	0.61
Q117	G	2.08	0.79	0.97
Q117	H	2.33	1.12	1.12

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
Q117	M	1.54	1.89	0.87
Q117	P	-0.25	1.13	0.61
Q117	Q	1.00	1.00	1.00
Q117	R	1.56	1.05	1.00
Q117	S	1.95	0.87	1.13
Q117	T	2.23	1.10	1.06
Q117	V	2.15	0.76	0.67
Q117	W	2.16	0.71	0.57
Q117	Y	2.23	1.13	0.76
V118	A	0.84	0.85	1.20
V118	C	0.78	1.14	1.28
V118	D	-0.14	0.40	0.38
V118	E	-0.14	-0.43	0.37
V118	F	0.86	1.00	0.89
V118	G	1.08	0.56	0.67
V118	I	0.96	0.55	1.01
V118	K	1.13	-2.50	0.28
V118	L	0.93	1.05	0.93
V118	M	0.60	0.93	0.90
V118	P	0.12	0.22	0.52
V118	Q	0.38	1.50	0.57
V118	R	0.36	0.07	0.46
V118	S	0.95	0.82	0.96
V118	T	0.99	0.92	0.90
V118	V	1.00	1.00	1.00
V118	W	0.83	-1.28	0.42
V118	Y	1.25	1.34	0.60
L119	A	0.81	1.02	1.18
L119	C	0.76	0.24	1.18
L119	D	0.24	0.28	0.97
L119	E	0.45	0.32	1.04
L119	F	0.56	-0.61	0.93
L119	G	0.93	-0.06	0.97
L119	H	0.91	0.46	0.89
L119	I	0.90	0.43	1.06
L119	L	1.00	1.00	1.00

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L119	N	0.58	0.11	1.14
L119	P	-0.14	-0.01	0.71
L119	R	0.43	-0.66	1.00
L119	S	0.83	-0.17	1.05
L119	T	0.97	0.10	0.94
L119	V	0.89	0.15	1.04
L119	W	0.77	0.20	0.88
L119	Y	0.77	0.56	0.89
T120	A	0.25	0.66	1.09
T120	C	0.75	0.92	1.14
T120	E	0.58	1.53	1.19
T120	H	0.88	0.50	1.07
T120	I	0.91	1.56	1.00
T120	K	0.87	1.09	1.12
T120	L	0.80	1.26	1.00
T120	M	0.05	1.22	0.98
T120	N	0.37	1.42	1.10
T120	P	0.07	-0.45	0.82
T120	Q	0.26	0.78	1.05
T120	R	0.24	0.60	0.99
T120	S	1.09	1.07	1.35
T120	T	1.00	1.00	1.00
T120	V	0.26	1.07	0.93
T120	Y	0.57	1.61	1.01
S121	A	1.12	1.55	1.10
S121	C	1.18	1.64	1.09
S121	E	0.89	1.04	1.01
S121	G	1.20	0.99	1.07
S121	K	1.24	0.78	1.04
S121	L	1.35	1.49	1.12
S121	N	1.14	1.06	1.17
S121	P	0.83	0.38	0.92
S121	Q	0.92	1.09	1.01
S121	R	1.26	0.70	1.06
S121	S	1.00	1.00	1.00
S121	T	1.13	1.26	0.93
S121	V	1.12	1.59	0.97
S121	W	1.33	0.77	0.91
A122	A	1.00	1.00	1.00
A122	D	0.26	0.06	0.77
A122	E	0.71	0.47	1.04
A122	F	0.97	0.15	0.87
A122	G	0.93	-0.42	0.85
A122	H	1.14	0.17	1.00
A122	I	1.13	0.65	1.04
A122	K	1.08	0.45	0.96
A122	L	0.93	1.02	1.07
A122	M	0.81	0.94	1.06
A122	N	0.83	0.70	1.11
A122	P	0.61	0.55	1.07
A122	Q	0.69	0.74	1.02
A122	R	0.71	0.40	0.94
A122	S	1.03	0.43	1.05
A122	T	1.08	0.52	0.97
A122	V	1.04	0.89	1.05
A122	W	0.99	0.86	0.88
G123	A	0.89	1.19	0.96
G123	C	0.95	0.30	0.92
G123	D	1.73	0.84	0.90
G123	E	1.13	0.56	0.96
G123	F	0.84	0.80	0.85
G123	G	1.00	1.00	1.00
G123	H	1.00	0.74	0.84
G123	K	0.97	1.12	0.93
G123	L	0.99	1.38	0.79
G123	M	0.84	1.38	0.85
G123	N	0.89	0.71	0.92
G123	P	1.32	0.81	0.89
G123	Q	0.01	0.31	0.37
G123	R	0.66	0.60	0.83
G123	T	1.06	0.54	0.85
G123	V	1.40	0.59	0.89

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
G123	W	0.95	1.39	0.77
G123	Y	0.96	1.24	0.87
G124	A	0.84	0.03	1.20
G124	C	0.72	0.67	1.07
G124	D	0.76	0.64	0.99
G124	F	1.32	0.95	0.70
G124	G	1.00	1.00	1.00
G124	H	1.59	-0.10	0.98
G124	I	1.85	-0.08	0.92
G124	L	1.92	0.54	0.98
G124	M	0.97	-0.05	1.36
G124	N	0.98	0.60	1.18
G124	P	-0.11	-0.08	0.37
G124	Q	1.12	0.21	1.02
G124	R	1.14	0.41	0.88
G124	S	1.27	0.56	1.00
G124	T	1.64	0.32	0.97
G124	V	1.44	0.33	0.93
G124	W	0.73	-0.31	0.84
G124	Y	1.23	0.56	0.66
V125	A	1.69	0.93	0.91
V125	C	0.96	0.54	0.67
V125	D	1.24	0.54	0.76
V125	E	0.81	0.39	0.73
V125	F	0.96	0.63	0.77
V125	G	2.95	1.09	0.60
V125	I	1.01	0.94	1.05
V125	P	1.50	0.62	0.83
V125	R	1.30	0.47	0.82
V125	S	1.94	0.79	0.75
V125	V	1.00	1.00	1.00
V125	W	0.37	0.25	0.48
V125	Y	1.08	0.81	0.82
G126	A	0.96	0.55	1.02
G126	C	0.35	0.98	0.96
G126	D	0.33	1.22	0.93
G126	E	0.67	0.60	1.02
G126	G	1.00	1.00	1.00
G126	I	0.84	0.01	0.81
G126	L	1.17	0.54	0.90
G126	M	0.43	1.17	0.92
G126	N	0.38	0.85	1.04
G126	P	1.17	0.67	0.82
G126	R	0.43	0.76	0.89
G126	S	0.76	0.90	0.90
G126	T	1.58	0.74	0.90
G126	V	0.89	0.18	0.84
G126	Y	0.54	0.23	0.82
T127	A	0.73	1.10	1.10
T127	C	0.76	0.65	1.04
T127	D	0.46	0.62	1.03
T127	E	0.40	-0.01	1.03
T127	G	0.95	0.71	1.04
T127	H	1.57	0.60	0.99
T127	I	1.06	0.20	0.91
T127	L	0.90	-0.03	0.94
T127	M	0.79	0.64	1.02
T127	P	0.14	0.77	0.95
T127	Q	0.55	0.15	0.86
T127	S	1.05	0.83	1.08
T127	T	1.00	1.00	1.00
T127	V	1.07	0.68	1.06
T128	A	0.76	1.31	1.23
T128	D	0.78	0.66	1.14
T128	F	0.79	1.71	1.01
T128	H	0.99	1.08	1.19
T128	K	1.06	1.57	1.10
T128	L	1.06	1.72	0.97
T128	M	0.72	1.06	1.28
T128	N	0.70	1.36	1.29
T128	P	0.87	1.16	1.18
T128	Q	0.78	1.34	1.24

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T128	R	0.87	1.70	1.03
T128	S	0.92	1.27	1.07
T128	T	1.00	1.00	1.00
T128	V	0.98	1.15	1.05
T128	W	0.92	1.23	0.95
T128	Y	0.95	1.81	0.96
Y129	A	0.64	0.17	1.39
Y129	C	0.66	0.61	1.42
Y129	D	0.35	0.23	1.35
Y129	F	0.71	0.71	1.44
Y129	G	0.39	-0.56	1.10
Y129	K	0.31	-0.29	1.00
Y129	L	0.78	0.27	1.22
Y129	M	0.68	0.21	1.28
Y129	N	0.46	0.53	1.24
Y129	P	0.15	0.59	1.11
Y129	R	0.38	0.18	1.00
Y129	S	0.67	0.69	1.08
Y129	T	0.46	0.14	1.00
Y129	V	0.24	-0.29	1.00
Y129	W	0.47	-0.15	1.01
Y129	Y	1.00	1.00	1.00
P130	A	0.82	0.44	1.03
P130	C	0.95	0.64	0.93
P130	E	1.00	0.22	1.08
P130	F	1.08	0.48	0.89
P130	G	1.16	-0.19	1.11
P130	H	1.17	0.01	1.00
P130	I	1.12	0.41	0.94
P130	K	1.16	0.55	1.05
P130	L	1.12	0.09	0.98
P130	M	0.66	0.76	1.03
P130	P	1.00	1.00	1.00
P130	R	1.11	0.53	0.95
P130	S	1.16	-0.14	0.96
P130	T	1.19	-0.06	0.96

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P130	V	1.15	0.37	0.94
P130	W	1.15	0.28	0.80
A131	A	1.00	1.00	1.00
A131	D	1.31	0.40	0.80
A131	E	1.36	0.97	0.88
A131	G	1.66	0.87	0.83
A131	H	1.72	0.82	0.75
A131	L	1.83	0.59	0.73
A131	P	1.52	0.71	0.94
A131	Q	1.29	0.74	0.69
A131	R	1.76	1.04	0.61
A131	S	1.48	0.68	0.87
A131	V	1.59	0.78	0.89
A131	W	1.61	-0.42	0.65
A131	Y	1.50	0.48	0.73
P132	A	0.49	6.08	0.94
P132	C	0.49	5.68	0.94
P132	D	-0.11	-7.16	0.62
P132	E	0.19	3.02	0.80
P132	F	0.76	-1.33	0.49
P132	G	0.83	4.98	0.79
P132	H	0.50	-1.95	0.68
P132	I	0.58	-3.19	0.64
P132	L	0.87	2.24	0.67
P132	N	0.30	1.05	0.83
P132	P	0.09	6.91	1.03
P132	Q	0.41	6.15	0.91
P132	R	0.02	-2.19	0.65
P132	S	1.13	5.05	0.96
P132	T	0.85	-2.01	0.75
P132	V	0.85	-2.29	0.78
P132	W	0.77	-2.64	0.37
P132	Y	1.57	4.78	0.60
K133	A	0.67	0.10	1.01
K133	C	0.56	-0.11	0.72
K133	E	0.63	0.76	1.01

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
K133	F	0.86	0.59	0.73
K133	G	0.97	0.31	0.87
K133	H	1.02	0.31	0.87
K133	I	0.89	0.45	0.78
K133	K	1.00	1.00	1.00
K133	L	1.05	1.92	0.76
K133	M	0.68	0.33	0.98
K133	P	0.39	0.71	0.89
K133	Q	0.69	0.52	1.13
K133	R	0.78	0.83	1.01
K133	S	0.84	0.58	1.02
K133	T	0.93	0.39	0.97
K133	V	0.90	0.23	0.87
K133	W	0.97	0.99	0.46
K133	Y	1.12	1.44	0.75
V134	A	0.75	1.64	0.87
V134	C	0.77	1.37	0.91
V134	D	-0.08	-0.08	-0.02
V134	G	1.71	1.42	0.45
V134	I	1.12	0.89	0.99
V134	K	-0.08	-0.08	-0.02
V134	L	1.13	1.45	0.78
V134	M	0.82	1.89	0.83
V134	N	1.18	2.80	0.25
V134	P	-0.08	1.71	0.43
V134	Q	0.04	0.79	0.44
V134	R	-0.08	-0.08	-0.02
V134	S	1.16	1.44	0.62
V134	T	1.25	0.86	0.82
V134	V	1.00	1.00	1.00
V134	W	-0.08	-0.08	-0.02
V134	Y	-0.08	-0.08	-0.02
L135	D	-0.13	2.90	0.27
L135	E	-0.13	0.63	0.39
L135	F	0.34	-0.03	0.45
L135	G	0.33	-1.71	0.28

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L135	K	0.66	-1.23	0.28
L135	L	1.00	1.00	1.00
L135	M	0.77	0.78	1.01
L135	P	-0.13	-1.31	0.22
L135	Q	0.34	0.17	0.66
L135	R	0.06	-1.41	0.25
L135	S	0.50	-0.65	0.44
L135	T	0.73	-0.42	0.50
L135	V	0.83	0.43	0.82
L135	W	0.71	-0.42	0.36
V136	A	0.60	1.60	0.66
V136	C	0.57	1.23	0.87
V136	E	-0.09	0.20	0.25
V136	L	0.98	1.13	1.03
V136	N	-0.09	0.40	0.26
V136	P	-0.09	-0.12	0.52
V136	R	-0.09	-0.12	-0.02
V136	T	1.13	1.13	0.68
V136	V	1.00	1.00	1.00
V136	W	-0.09	-0.12	-0.02
V137	A	1.07	1.46	0.64
V137	C	0.98	1.42	0.85
V137	D	-0.17	-0.23	-0.01
V137	E	-0.17	-0.23	-0.01
V137	F	-0.17	-0.23	-0.01
V137	G	1.02	0.26	0.13
V137	I	0.98	0.70	0.83
V137	L	1.09	1.27	0.82
V137	M	1.22	1.13	0.89
V137	N	0.46	-1.29	0.15
V137	P	-0.17	-0.23	-0.01
V137	R	-0.17	-0.23	-0.01
V137	S	0.96	0.29	0.50
V137	T	1.08	0.93	0.73
V137	V	1.00	1.00	1.00
V137	W	-0.17	-0.23	-0.01

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
V137	Y	-0.17	-0.23	-0.01
S138	A	0.69	1.28	1.44
S138	C	0.64	1.18	1.17
S138	E	-0.13	-0.19	-0.02
S138	F	-0.13	-0.19	-0.02
S138	G	1.05	1.11	1.09
S138	H	-0.13	-0.19	-0.02
S138	I	1.15	0.35	0.56
S138	L	-0.13	-0.19	-0.02
S138	M	-0.13	-0.19	-0.02
S138	N	0.62	1.31	0.77
S138	P	0.54	1.39	0.45
S138	Q	-0.13	-0.19	-0.02
S138	R	-0.13	-0.19	-0.02
S138	S	1.00	1.00	1.00
S138	V	1.00	0.69	0.67
S138	W	-0.13	-0.19	-0.02
S138	Y	-0.13	-0.19	-0.02
P139	C	0.08	-0.12	0.18
P139	D	-0.13	-1.44	0.15
P139	E	-0.13	-5.11	0.19
P139	F	-0.13	-4.13	0.16
P139	G	0.50	-3.08	0.23
P139	H	-0.13	-6.03	0.19
P139	I	-0.13	-3.71	0.21
P139	K	-0.13	-4.09	0.12
P139	L	-0.13	-0.17	-0.02
P139	N	-0.13	-2.11	0.16
P139	P	1.00	1.00	1.00
P139	Q	-0.13	-0.32	0.18
P139	R	0.37	-1.04	0.23
P139	S	0.88	-0.52	0.43
P139	T	0.01	-3.48	0.15
P139	V	-0.13	-1.70	0.17
P139	W	-0.13	-0.17	-0.02
P139	Y	-0.13	-0.17	-0.02

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P140	A	1.90	1.83	0.61
P140	C	0.39	1.07	0.40
P140	D	-0.45	-0.23	-0.02
P140	F	-0.45	2.89	0.19
P140	G	0.96	3.11	0.20
P140	H	0.59	2.25	0.23
P140	I	0.45	-1.03	0.24
P140	K	-0.45	-0.23	-0.02
P140	L	-0.45	-0.23	-0.02
P140	M	-0.45	-0.23	-0.02
P140	P	1.00	1.00	1.00
P140	Q	-0.45	-1.32	0.32
P140	R	-0.45	-2.74	0.25
P140	S	1.31	-1.22	0.43
P140	T	1.74	-0.78	0.29
P140	V	0.50	-1.12	0.34
P140	W	0.50	-0.97	0.17
P140	Y	0.32	-1.90	0.24
P141	A	1.10	1.08	1.13
P141	G	1.64	-0.05	1.02
P141	H	2.07	0.79	0.93
P141	I	2.29	0.38	0.90
P141	L	2.32	0.65	0.74
P141	N	1.32	0.97	0.96
P141	P	1.00	1.00	1.00
P141	Q	1.39	0.37	0.88
P141	R	1.65	-0.26	0.61
P141	S	1.70	0.02	0.90
P141	T	1.84	0.12	0.82
P141	V	1.96	0.16	0.72
L142	A	0.80	0.56	0.67
L142	C	0.74	0.70	0.78
L142	D	-0.12	-0.13	-0.01
L142	F	1.05	0.54	0.46
L142	G	-0.12	-0.13	-0.01
L142	I	0.64	0.28	1.05

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
L142	K	1.60	0.66	0.23
L142	L	1.00	1.00	1.00
L142	M	-0.12	-0.13	-0.01
L142	N	-0.12	-0.13	-0.01
L142	P	0.54	0.44	0.48
L142	Q	0.67	0.33	0.49
L142	R	-0.12	-0.13	-0.01
L142	S	0.84	0.31	0.65
L142	T	-0.12	-0.13	-0.01
L142	V	0.84	0.33	0.82
L142	W	2.41	-1.89	0.16
A143	A	1.00	1.00	1.00
A143	C	1.39	1.07	0.81
A143	D	1.45	1.22	0.71
A143	E	1.43	1.13	0.71
A143	F	1.56	0.68	0.99
A143	G	1.48	0.42	1.17
A143	H	2.90	1.36	0.70
A143	K	3.16	1.37	0.62
A143	L	2.51	1.28	0.71
A143	N	1.30	0.82	0.79
A143	P	1.53	0.39	0.63
A143	Q	1.74	0.81	0.72
A143	R	2.15	0.99	0.62
A143	S	1.77	0.63	0.98
A143	T	2.18	0.97	0.74
A143	V	2.45	0.99	0.81
A143	W	2.27	-0.21	0.37
P144	A	1.09	0.79	0.91
P144	D	1.45	1.38	0.60
P144	F	1.82	1.08	0.66
P144	G	1.45	0.62	0.78
P144	H	1.94	1.60	0.66
P144	K	2.09	1.09	0.67
P144	L	1.43	1.15	0.86
P144	M	1.24	1.01	0.76

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P144	N	1.44	1.49	0.74
P144	P	1.00	1.00	1.00
P144	Q	1.37	1.08	0.77
P144	R	1.76	1.14	0.68
P144	S	1.69	0.92	0.77
P144	T	1.46	0.81	0.80
P144	Y	2.34	1.65	0.70
M145	A	0.44	0.79	0.94
M145	C	1.02	0.93	0.94
M145	E	0.28	0.48	0.74
M145	F	1.49	0.77	0.95
M145	G	0.48	0.26	0.92
M145	I	0.79	0.53	1.16
M145	L	1.72	0.61	1.07
M145	M	1.00	1.00	1.00
M145	P	0.64	0.78	0.78
M145	Q	0.68	0.57	0.86
M145	R	1.15	0.69	0.78
M145	S	0.64	0.78	0.91
M145	T	1.01	0.79	0.91
M145	V	0.72	0.63	1.00
M145	W	1.15	-0.13	0.49
M145	Y	0.94	0.82	0.68
P146	A	0.20	1.36	0.73
P146	C	0.31	1.69	0.62
P146	F	0.55	1.53	0.51
P146	G	0.24	1.04	0.51
P146	H	0.50	1.57	0.56
P146	L	0.56	2.00	0.53
P146	M	0.39	1.23	0.79
P146	N	0.37	1.00	0.78
P146	P	1.00	1.00	1.00
P146	R	0.36	1.06	0.66
P146	S	0.46	0.96	0.82
P146	T	0.38	0.76	0.80
P146	V	0.55	0.77	0.89

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Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
P146	W	0.56	0.68	0.64
P146	Y	0.35	1.44	0.54
H147	A	1.28	0.98	0.96
H147	C	0.94	1.17	1.04
H147	D	0.95	1.18	1.00
H147	E	1.11	1.10	0.96
H147	G	-0.12	-0.15	-0.02
H147	H	1.00	1.00	1.00
H147	I	0.89	0.92	0.89
H147	K	0.94	1.06	0.89
H147	L	0.69	1.29	1.09
H147	M	0.73	1.44	0.86
H147	N	0.84	1.25	0.98
H147	P	1.12	1.21	0.71
H147	Q	0.71	1.03	0.86
H147	R	0.89	0.94	0.69
H147	S	1.26	0.75	0.92
H147	T	1.20	0.84	0.85
H147	V	0.96	0.92	0.90
H147	W	0.88	1.05	0.79
H147	Y	0.75	1.12	0.94
P148	A	1.64	1.06	0.96
P148	D	1.03	1.34	0.74
P148	E	1.42	1.19	0.76
P148	F	1.37	1.50	0.64
P148	G	0.87	1.20	0.70
P148	K	1.79	1.30	0.72
P148	L	1.64	1.39	0.74
P148	P	1.00	1.00	1.00
P148	Q	1.33	0.98	0.81
P148	R	1.51	1.25	0.79
P148	S	1.46	1.21	0.74
P148	T	1.50	1.09	0.79
P148	V	2.43	1.04	0.76
P148	Y	1.46	1.37	0.72
W149	A	0.21	0.31	1.35

Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
W149	C	0.18	0.12	0.93
W149	E	0.00	-0.04	0.85
W149	F	0.53	0.50	1.27
W149	G	0.26	0.45	1.39
W149	H	0.60	1.01	0.81
W149	I	0.21	0.24	0.83
W149	L	0.30	0.64	1.06
W149	M	0.33	0.49	1.32
W149	P	-0.32	-0.16	0.92
W149	Q	0.11	0.40	1.10
W149	R	0.04	-0.32	0.67
W149	S	0.16	0.33	1.28
W149	T	0.26	0.44	0.84
W149	W	1.00	1.00	1.00
W149	Y	0.58	0.75	1.15
F150	A	0.01	0.54	1.70
F150	C	0.43	0.78	1.41
F150	E	1.23	0.73	1.32
F150	F	1.00	1.00	1.00
F150	G	0.14	0.46	1.13
F150	H	0.53	1.18	1.09
F150	I	0.40	0.78	1.19
F150	K	0.41	0.85	1.33
F150	L	1.29	1.30	1.14
F150	M	0.80	0.63	1.69
F150	N	0.55	0.36	1.52
F150	P	0.18	0.32	1.38
F150	T	0.37	0.58	1.27
F150	V	0.22	0.51	1.26
F150	W	0.19	0.62	1.26
F150	Y	0.72	1.07	1.24
Q151	A	1.29	2.93	0.46
Q151	C	1.05	2.55	0.38
Q151	D	1.47	2.81	0.83
Q151	E	1.14	2.07	0.99
Q151	F	0.31	-8.08	0.21

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
Q151	H	1.06	2.19	0.94
Q151	I	0.08	-2.76	0.16
Q151	K	1.07	2.19	1.04
Q151	L	0.40	-1.53	0.17
Q151	M	1.24	6.36	0.24
Q151	P	1.35	1.91	0.50
Q151	Q	1.00	1.00	1.00
Q151	R	1.36	2.32	0.68
Q151	S	1.05	2.25	0.86
Q151	T	1.24	2.37	0.64
Q151	V	0.36	-1.65	0.25
Q151	W	0.77	0.32	0.33
Q151	Y	1.01	2.75	0.41
L152	A	0.88	1.29	0.85
L152	C	1.00	1.14	0.87
L152	D	1.07	0.86	0.81
L152	E	1.08	1.23	0.93
L152	G	1.08	0.77	0.85
L152	H	1.09	0.92	0.93
L152	I	1.04	0.61	0.77
L152	K	1.21	0.91	0.93
L152	L	1.00	1.00	1.00
L152	M	0.99	1.10	0.82
L152	P	0.81	0.61	0.54
L152	Q	1.07	0.76	0.84
L152	R	1.20	0.91	0.89
L152	S	1.12	0.84	0.84
L152	T	1.12	0.69	0.82
L152	V	1.22	0.88	0.83
L152	W	1.18	1.55	0.74
L152	Y	1.09	1.37	0.89
I153	A	1.19	1.49	0.76
I153	F	1.23	1.75	0.47
I153	H	1.46	2.00	0.56
I153	I	1.00	1.00	1.00
I153	K	1.62	2.44	0.43
I153	L	1.27	1.50	0.82
I153	N	0.72	0.89	1.04
I153	P	0.25	1.87	0.31
I153	S	0.87	1.66	0.61
I153	T	1.27	1.62	0.64
I153	V	0.96	1.15	0.78
F154	D	-0.19	-1.06	-0.02
F154	E	-0.19	-1.06	-0.02
F154	F	1.00	1.00	1.00
F154	G	-0.19	-0.64	0.17
F154	L	-0.19	-1.06	-0.02
F154	P	-0.19	-1.06	-0.02
F154	Q	0.39	0.97	0.45
F154	S	0.13	0.29	0.35
F154	T	0.12	-1.76	0.19
F154	V	-0.19	-14.19	0.18
F154	Y	1.32	4.96	0.92
E155	A	0.99	2.59	0.83
E155	D	1.08	1.24	0.89
E155	E	1.00	1.00	1.00
E155	F	1.07	0.23	0.60
E155	G	1.17	1.12	0.82
E155	I	0.95	0.65	0.61
E155	K	1.23	1.33	0.83
E155	L	1.31	2.07	0.60
E155	M	0.73	2.91	0.74
E155	N	0.79	1.79	0.86
E155	P	0.79	2.60	0.65
E155	Q	0.90	0.69	0.87
E155	R	1.47	-0.07	0.71
E155	S	1.08	1.12	0.82
E155	T	1.49	1.19	0.76
E155	V	0.79	0.47	0.63
E155	Y	1.27	2.65	0.55
G156	A	0.99	1.21	0.88
G156	C	1.07	1.37	0.84

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
G156	D	0.96	1.62	0.93
G156	E	0.94	1.14	0.91
G156	F	0.90	0.73	0.78
G156	G	1.00	1.00	1.00
G156	H	1.04	1.40	0.84
G156	I	0.70	-0.08	0.44
G156	K	1.10	1.11	0.88
G156	L	0.90	0.94	0.74
G156	M	1.09	1.62	0.80
G156	N	1.07	1.38	0.97
G156	P	1.44	1.29	0.59
G156	R	1.05	1.21	0.80
G156	S	1.02	1.04	0.88
G156	T	1.15	1.53	0.79
G156	V	0.88	0.97	0.58
G156	W	0.89	0.90	0.56
G156	Y	0.96	1.40	0.80
G157	A	0.77	0.87	1.00
G157	C	0.96	0.61	0.92
G157	D	0.93	0.94	0.41
G157	E	0.98	0.84	0.61
G157	F	1.27	1.42	0.61
G157	G	1.00	1.00	1.00
G157	H	1.14	1.57	0.70
G157	I	1.11	1.33	0.36
G157	K	1.28	1.47	0.46
G157	M	0.96	0.85	0.70
G157	P	0.86	0.01	0.31
G157	R	1.51	-0.10	0.42
G157	S	1.30	0.19	0.93
G157	T	1.74	0.99	0.68
G157	V	1.23	0.40	0.59
E158	A	1.45	1.28	0.91
E158	C	1.46	1.37	0.67
E158	D	1.35	0.89	0.82
E158	E	1.00	1.00	1.00

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
E158	F	2.06	1.77	0.46
E158	H	2.40	1.01	0.59
E158	I	1.38	0.94	0.76
E158	K	2.08	1.88	0.62
E158	L	1.59	1.96	0.70
E158	M	1.39	1.73	0.71
E158	N	1.41	1.58	0.82
E158	P	1.41	1.19	0.85
E158	Q	1.49	1.24	0.85
E158	R	1.99	1.29	0.62
E158	S	1.57	1.27	0.82
E158	T	1.45	0.91	0.77
E158	V	1.52	0.89	0.81
E158	W	1.77	1.31	0.67
E158	Y	1.77	2.48	0.57
Q159	A	1.08	0.28	1.13
Q159	C	1.13	0.31	0.79
Q159	D	1.09	0.63	0.90
Q159	E	0.99	0.97	1.14
Q159	G	0.96	0.72	1.03
Q159	H	0.96	1.48	0.90
Q159	L	1.02	0.70	0.83
Q159	M	1.07	0.84	0.83
Q159	P	1.06	0.49	0.81
Q159	Q	1.00	1.00	1.00
Q159	R	1.15	0.74	0.76
Q159	S	1.10	0.73	0.81
K160	A	0.39	1.14	0.86
K160	C	0.48	1.29	0.77
K160	D	-0.15	1.19	0.40
K160	G	0.91	0.30	0.56
K160	H	0.98	0.57	0.65
K160	I	0.97	1.00	0.78
K160	K	1.00	1.00	1.00
K160	L	0.97	0.95	0.77
K160	M	0.31	1.47	0.78

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
K160	N	0.37	1.12	0.65
K160	P	-0.15	1.66	0.31
K160	Q	0.45	1.41	0.75
K160	R	0.83	1.15	0.76
K160	S	0.85	0.70	0.74
K160	W	0.89	-0.34	0.21
T161	C	0.84	0.56	1.01
T161	D	-0.14	-0.21	-0.02
T161	E	-0.14	-0.21	-0.02
T161	G	0.92	0.43	0.94
T161	H	1.82	-0.15	0.42
T161	I	1.40	0.98	0.91
T161	L	1.25	1.16	0.81
T161	M	0.57	1.72	0.83
T161	N	0.80	-0.86	0.32
T161	P	-0.14	-0.21	-0.02
T161	Q	1.04	1.50	0.90
T161	R	3.61	-1.68	0.42
T161	S	0.92	0.57	0.98
T161	T	1.00	1.00	1.00
T161	V	1.27	1.24	1.00
T161	W	1.41	0.00	0.52
T161	Y	2.40	2.62	0.23
T162	C	0.95	3.57	1.17
T162	F	0.99	3.23	1.05
T162	G	1.00	1.82	0.88
T162	H	1.02	3.91	1.08
T162	I	0.99	2.21	1.16
T162	K	1.22	3.13	0.98
T162	L	1.00	3.59	1.05
T162	M	0.77	3.49	0.89
T162	N	0.83	3.84	0.98
T162	P	0.96	4.37	0.81
T162	Q	0.93	2.45	0.89
T162	R	1.17	1.23	0.80
T162	S	0.98	2.01	0.97
T162	T	1.00	1.00	1.00
T162	W	1.15	2.04	0.85
T162	Y	1.03	2.89	1.03
E163	A	1.11	1.79	0.73
E163	C	1.11	1.08	0.67
E163	D	0.90	1.08	0.82
E163	E	1.00	1.00	1.00
E163	F	1.07	0.27	0.49
E163	G	1.25	0.80	0.79
E163	H	1.32	0.82	0.69
E163	L	1.50	1.94	0.58
E163	N	0.91	1.00	0.77
E163	P	0.08	0.77	0.30
E163	R	1.12	0.49	0.72
E163	S	1.12	0.85	0.81
E163	V	1.13	0.55	0.69
E163	W	1.21	0.98	0.49
E163	Y	1.41	1.89	0.60
L164	A	-0.14	-0.85	0.21
L164	C	0.09	0.91	0.63
L164	D	-0.14	-0.85	0.12
L164	E	-0.14	-0.48	0.18
L164	F	0.50	0.86	0.94
L164	G	-0.14	-0.14	0.19
L164	H	0.02	0.12	0.16
L164	L	1.00	1.00	1.00
L164	M	0.69	1.26	1.09
L164	N	-0.14	1.31	0.26
L164	P	-0.14	2.41	0.17
L164	Q	-0.14	1.01	0.24
L164	R	-0.14	1.61	0.17
L164	S	0.32	1.11	0.25
L164	T	0.82	0.99	0.52
L164	V	0.87	1.02	1.08
L164	Y	0.43	-1.28	0.20
A165	A	1.00	1.00	1.00

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Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
A165	C	0.99	1.42	0.97
A165	D	0.89	1.69	0.62
A165	F	1.23	1.00	0.74
A165	G	1.05	1.07	1.14
A165	I	1.17	0.59	0.64
A165	K	1.35	0.82	0.78
A165	L	1.08	1.55	0.70
A165	M	0.97	1.56	0.77
A165	N	1.01	1.20	0.91
A165	P	1.14	1.34	0.91
A165	Q	1.21	1.32	1.05
A165	R	1.70	1.29	0.87
A165	S	1.00	0.94	1.05
A165	T	1.18	1.32	0.83
A165	V	1.21	1.13	0.88
A165	Y	1.20	0.84	0.67
R166	A	0.73	1.51	1.12
R166	D	0.56	1.55	1.16
R166	F	1.00	1.10	0.85
R166	G	1.15	0.91	1.19
R166	H	1.20	1.56	0.97
R166	I	1.26	1.39	0.86
R166	K	1.17	1.20	1.19
R166	L	1.27	1.50	1.08
R166	M	0.65	1.29	1.26
R166	N	0.75	1.21	1.16
R166	P	0.43	1.50	0.97
R166	R	1.00	1.00	1.00
R166	S	1.16	0.95	0.98
R166	T	1.19	0.74	1.04
R166	V	1.17	0.76	0.94
R166	W	1.25	1.08	0.80
R166	Y	1.29	1.22	0.85
V167	A	0.56	4.99	0.98
V167	C	0.79	5.37	1.01
V167	D	0.56	5.54	0.98

Table 10-12. Performance Indices				
Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
V167	G	0.99	2.83	1.08
V167	H	1.03	2.11	1.12
V167	I	1.08	1.00	1.04
V167	L	0.84	2.56	1.13
V167	M	0.53	3.84	1.04
V167	P	0.31	6.08	0.85
V167	Q	0.55	2.41	0.97
V167	R	0.78	2.25	0.88
V167	S	0.96	1.86	1.04
V167	T	1.13	2.47	0.96
V167	V	1.00	1.00	1.00
V167	Y	1.07	2.15	0.94
Y168	C	0.69	-4.73	0.57
Y168	D	-0.11	-1.98	-0.03
Y168	E	-0.11	-1.98	-0.03
Y168	F	0.68	5.17	1.28
Y168	G	1.89	-40.74	0.23
Y168	H	-0.11	-1.98	-0.03
Y168	I	0.83	-0.59	0.90
Y168	K	-0.11	-1.98	-0.03
Y168	L	0.59	5.39	1.27
Y168	N	-0.11	-1.98	-0.03
Y168	P	-0.11	-1.98	-0.03
Y168	Q	0.28	-8.27	0.25
Y168	R	-0.11	-1.98	-0.03
Y168	S	-0.11	-1.98	-0.03
Y168	T	1.51	-22.96	0.39
Y168	V	1.19	-12.96	0.57
Y168	W	-0.11	-1.98	-0.03
Y168	Y	1.00	1.00	1.00
S169	A	0.94	1.13	0.95
S169	C	1.03	1.38	0.78
S169	I	1.16	1.53	0.66
S169	K	1.21	1.27	0.94
S169	L	1.08	1.47	0.82
S169	M	0.86	1.40	0.86

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
S169	P	0.87	0.89	0.69
S169	Q	1.02	1.37	0.88
S169	R	1.24	1.19	0.77
S169	S	1.00	1.00	1.00
S169	T	1.15	0.97	0.82
S169	Y	1.26	1.10	0.77
A170	A	1.00	1.00	1.00
A170	C	1.15	1.06	1.02
A170	D	1.27	1.32	0.88
A170	E	1.28	1.17	0.99
A170	F	1.44	1.17	0.83
A170	G	1.59	0.62	0.96
A170	I	1.59	0.44	0.95
A170	K	1.71	0.83	0.96
A170	L	1.05	0.85	0.87
A170	M	1.03	1.28	0.93
A170	N	1.21	1.17	0.96
A170	P	0.75	1.33	0.80
A170	Q	1.15	0.89	0.98
A170	S	1.47	0.47	0.99
A170	T	1.40	0.72	0.86
A170	V	1.20	0.74	0.83
A170	W	1.04	0.83	0.82
A170	Y	0.80	0.89	0.89
L171	A	0.35	1.66	0.79
L171	C	0.56	1.73	0.97
L171	D	-0.06	-0.13	-0.01
L171	F	1.30	1.97	0.87
L171	G	1.26	1.33	0.50
L171	H	1.67	1.07	0.61
L171	I	1.53	1.42	1.16
L171	K	2.05	1.53	0.31
L171	L	1.00	1.00	1.00
L171	M	0.53	2.22	0.90
L171	N	0.96	2.79	0.40
L171	Q	0.97	1.93	0.67
L171	R	0.71	-0.20	0.24
L171	S	1.43	1.76	0.72
L171	T	1.54	1.36	0.80
L171	V	1.02	1.39	0.92
L171	Y	1.20	1.35	0.88
A172	A	1.00	1.00	1.00
A172	C	1.20	0.86	1.09
A172	D	-0.15	1.42	0.16
A172	E	-0.15	-0.44	0.19
A172	G	1.41	0.84	1.07
A172	I	1.70	0.58	0.30
A172	K	0.95	-0.43	0.17
A172	L	1.20	1.22	0.70
A172	M	0.84	1.06	0.84
A172	N	0.37	0.76	0.30
A172	P	-0.15	0.58	0.16
A172	Q	0.27	0.18	0.34
A172	R	0.44	-0.18	0.20
A172	S	1.59	0.85	0.96
A172	T	1.25	0.71	0.85
A172	V	1.40	0.39	0.53
A172	W	1.43	0.45	0.12
A172	Y	0.87	1.76	0.13
S173	A	0.81	2.72	0.95
S173	C	0.82	3.07	0.59
S173	E	0.78	2.65	0.90
S173	F	0.96	2.30	0.71
S173	H	1.07	1.49	0.95
S173	I	0.99	2.22	0.78
S173	K	1.17	3.01	0.91
S173	L	1.15	3.86	0.77
S173	M	0.80	3.01	0.84
S173	P	0.19	2.66	0.35
S173	R	1.09	2.47	0.82
S173	S	1.00	1.00	1.00
S173	T	1.06	1.29	0.89

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
S173	V	0.95	2.54	0.75
S173	W	1.16	3.67	0.67
S173	Y	1.19	3.54	0.81
F174	A	0.59	2.09	0.61
F174	C	1.32	0.48	0.65
F174	F	1.00	1.00	1.00
F174	G	1.60	0.91	0.85
F174	H	0.93	1.05	0.86
F174	K	0.86	1.17	0.76
F174	L	1.05	1.83	0.82
F174	M	0.91	2.20	0.55
F174	P	1.54	1.46	0.13
F174	Q	1.42	0.46	0.82
F174	R	0.70	0.52	0.95
F174	S	1.16	0.61	0.75
F174	T	0.80	0.64	0.62
F174	V	0.60	0.67	0.82
F174	W	0.96	-0.02	0.85
F174	Y	0.84	1.66	0.77
M175	A	0.70	0.66	0.95
M175	E	0.95	1.43	0.89
M175	G	2.04	0.75	0.67
M175	L	1.61	0.86	1.19
M175	M	1.00	1.00	1.00
M175	N	1.39	1.02	1.11
M175	P	-0.20	0.08	0.16
M175	Q	1.56	0.83	0.98
M175	R	1.55	0.86	1.02
M175	T	2.21	0.90	0.98
M175	V	1.93	0.81	1.00
M175	W	1.25	0.76	1.14
M175	Y	0.77	0.72	1.35
K176	A	0.42	1.19	0.84
K176	C	0.58	1.01	0.87
K176	D	0.62	1.18	0.74
K176	E	0.67	1.08	0.88

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
K176	F	0.36	1.28	0.31
K176	G	1.01	0.73	0.80
K176	K	1.00	1.00	1.00
K176	L	1.00	0.92	0.58
K176	M	0.56	1.33	0.74
K176	N	0.60	0.94	0.85
K176	P	0.01	0.78	0.27
K176	Q	0.59	0.97	1.02
K176	R	0.71	1.03	1.06
K176	S	0.76	0.72	0.93
K176	T	1.04	0.97	0.70
K176	V	1.04	1.33	0.71
K176	W	1.19	1.16	0.41
K176	Y	1.04	0.93	0.60
P178	A	0.31	4.39	0.96
P178	D	0.18	6.44	0.93
P178	E	0.40	4.15	1.05
P178	G	1.09	2.95	0.67
P178	K	1.34	1.70	0.73
P178	L	1.82	7.15	0.53
P178	M	0.53	3.87	0.78
P178	P	0.06	5.02	0.93
P178	Q	0.15	3.64	0.93
P178	S	0.62	3.06	0.95
P178	T	0.70	2.28	0.81
P178	V	0.67	2.70	0.78
P178	W	1.14	0.02	0.64
P178	Y	1.38	6.91	0.74
F179	A	-0.18	-0.22	-0.02
F179	E	0.02	1.80	0.20
F179	F	1.00	1.00	1.00
F179	G	0.03	1.16	0.36
F179	H	0.79	0.93	0.91
F179	L	1.15	1.89	0.43
F179	N	0.77	0.95	0.46
F179	P	-0.18	-0.22	-0.02

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
F179	Q	0.46	-0.87	0.46
F179	R	-0.18	-0.22	-0.02
F179	S	0.78	0.34	0.62
F179	V	0.70	1.17	0.69
F179	W	0.89	0.86	0.62
F179	Y	1.05	1.47	0.65
F180	A	0.03	2.70	0.27
F180	C	0.65	1.94	0.66
F180	E	-0.14	-0.55	-0.02
F180	F	1.00	1.00	1.00
F180	G	0.37	-5.96	0.20
F180	I	1.20	2.11	0.79
F180	K	1.08	-6.98	0.24
F180	L	1.30	2.13	0.86
F180	M	0.71	4.36	0.96
F180	N	-0.14	3.05	0.29
F180	Q	0.21	-1.87	0.36
F180	R	0.64	-3.57	0.26
F180	S	0.56	-2.05	0.29
F180	T	1.01	-0.68	0.33
F180	V	1.14	3.24	0.76
F180	W	1.11	1.81	0.90
F180	Y	1.12	2.99	0.84
D181	A	1.35	1.23	0.65
D181	C	1.09	0.85	0.56
D181	D	1.00	1.00	1.00
D181	E	1.10	0.72	0.78
D181	F	-0.15	-0.17	-0.01
D181	G	1.09	0.52	0.37
D181	H	-0.15	-0.17	-0.01
D181	I	-0.15	-0.17	-0.01
D181	K	1.33	0.47	0.41
D181	L	1.25	-0.16	0.16
D181	M	-0.15	-0.17	-0.01
D181	N	-0.15	-0.17	-0.01
D181	P	1.03	0.66	0.60

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
D181	Q	1.14	0.60	0.54
D181	R	1.23	0.22	0.45
D181	S	1.21	0.55	0.56
D181	T	1.02	-0.32	0.24
D181	V	0.88	-0.34	0.21
D181	W	1.26	-0.52	0.28
D181	Y	1.29	-0.25	0.25
A182	A	1.00	1.00	1.00
A182	C	0.97	0.99	1.03
A182	G	0.92	0.94	0.90
A182	H	-0.14	-0.18	-0.02
A182	I	0.89	-2.48	0.20
A182	K	-0.14	-0.18	-0.02
A182	L	-0.14	-0.18	-0.02
A182	M	-0.14	-0.18	-0.02
A182	N	-0.14	0.53	0.14
A182	P	-0.14	-1.13	0.12
A182	Q	0.03	-0.84	0.14
A182	R	0.25	-2.69	0.12
A182	S	0.87	0.85	0.90
A182	T	1.14	0.11	0.48
A182	W	-0.14	-0.18	-0.02
A182	Y	-0.14	-0.18	-0.02
G183	C	0.56	1.99	0.92
G183	D	0.30	0.99	0.62
G183	F	0.68	0.19	0.75
G183	G	1.00	1.00	1.00
G183	H	0.98	0.95	0.87
G183	L	0.82	1.50	0.47
G183	P	-0.18	1.02	0.33
G183	Q	0.66	-0.20	0.97
G183	R	0.92	1.09	0.90
G183	S	0.94	-0.08	1.08
G183	V	0.56	-2.47	0.57
G183	Y	0.97	1.45	0.79
S184	A	0.60	1.69	1.31

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
S184	C	0.81	2.39	1.14
S184	D	0.84	2.24	1.15
S184	E	0.94	1.86	1.39
S184	F	1.05	1.27	0.89
S184	G	0.99	0.82	1.15
S184	H	1.02	0.74	1.07
S184	I	0.92	1.21	0.96
S184	K	0.97	1.61	1.02
S184	L	0.80	2.00	0.98
S184	M	0.51	1.77	1.25
S184	N	0.64	1.93	1.03
S184	P	-0.15	0.85	0.40
S184	Q	0.89	1.16	1.09
S184	S	1.00	1.00	1.00
S184	T	1.04	0.60	0.94
S184	V	0.80	1.25	1.03
S184	Y	1.06	1.09	0.84
V185	C	0.65	0.83	0.96
V185	D	0.40	-2.49	0.21
V185	E	0.73	0.88	0.76
V185	F	1.02	1.20	0.83
V185	G	1.12	-3.67	0.47
V185	H	1.30	-0.58	0.71
V185	I	1.07	0.63	1.03
V185	K	1.37	0.79	0.66
V185	L	1.23	0.93	0.75
V185	M	0.39	1.46	0.77
V185	Q	0.77	1.41	0.73
V185	R	1.15	0.79	0.57
V185	S	1.09	0.53	0.75
V185	T	1.11	0.91	0.79
V185	V	1.00	1.00	1.00
V185	W	1.36	-0.44	0.53
V185	Y	1.37	0.58	0.65
I186	A	1.46	1.79	0.90
I186	D	-0.13	4.29	0.19

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
I186	F	1.01	0.76	0.77
I186	G	1.86	-5.42	0.35
I186	I	1.00	1.00	1.00
I186	K	-0.13	-0.36	-0.01
I186	L	1.17	1.14	0.84
I186	M	0.86	1.38	1.11
I186	P	-0.13	-2.95	0.25
I186	R	0.62	-6.69	0.25
I186	S	1.39	-0.21	0.65
I186	T	1.51	0.23	0.79
I186	V	1.28	0.48	0.93
I186	W	-0.13	-0.36	-0.01
I186	Y	-0.13	-0.36	-0.01
S187	A	0.51	1.72	0.86
S187	C	0.70	1.67	0.79
S187	D	0.59	1.40	0.82
S187	F	1.02	0.65	0.73
S187	G	1.03	1.46	0.88
S187	H	1.29	1.51	0.68
S187	I	1.38	1.58	0.78
S187	K	1.45	1.16	0.76
S187	L	1.37	1.46	0.75
S187	M	0.49	1.87	0.85
S187	N	0.59	1.59	0.90
S187	P	0.44	-0.31	0.78
S187	Q	0.63	0.35	0.94
S187	R	1.04	0.55	0.82
S187	S	1.00	1.00	1.00
S187	T	1.12	0.23	0.74
S187	V	1.23	0.58	0.89
S187	W	1.30	0.52	0.73
S187	Y	1.43	0.80	0.76
T188	A	0.97	0.95	1.40
T188	C	0.60	0.87	2.04
T188	D	-0.05	-0.14	-0.02
T188	E	0.24	1.97	0.44

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Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
T188	F	0.96	-0.20	0.63
T188	G	0.93	0.79	1.32
T188	H	1.11	-0.79	0.74
T188	I	1.13	0.10	1.85
T188	K	-0.05	-0.14	-0.02
T188	L	0.76	0.42	1.76
T188	M	0.49	0.75	1.60
T188	N	0.69	1.69	1.24
T188	P	-0.05	-0.14	-0.02
T188	Q	-0.05	-0.14	-0.02
T188	R	1.01	-0.47	1.41
T188	S	1.16	0.91	1.52
T188	T	1.00	1.00	1.00
T188	V	1.22	0.15	1.53
T188	W	-0.05	-0.14	-0.02
T188	Y	1.48	0.09	0.47
D189	A	0.05	1.18	0.53
D189	C	0.19	0.94	0.56
D189	D	0.03	0.89	0.90
D189	E	0.35	0.77	0.85
D189	F	0.83	0.37	0.63
D189	G	0.80	0.80	0.83
D189	H	1.25	0.95	0.78
D189	I	0.73	1.27	0.69
D189	L	1.30	1.30	0.61
D189	M	0.06	0.88	0.48
D189	N	0.22	0.57	0.80
D189	P	-0.12	0.97	0.67
D189	R	0.86	0.39	0.65
D189	S	0.88	0.81	0.85
D189	T	1.00	1.21	0.73
D189	V	0.73	0.71	0.72
D189	W	1.09	0.76	0.60
I194	A	0.29	0.00	1.15
I194	C	0.27	-0.02	1.17
I194	F	0.07	-0.03	0.95

Table 10-12. Performance Indices

Wild-Type Res./ Pos.	Mut.	PAF PI	PAD PI	Prot. PI
I194	G	0.10	0.04	0.34
I194	I	1.00	1.00	1.00
I194	L	0.80	0.58	1.32
I194	P	0.15	-1.42	0.16
I194	R	0.02	-0.40	0.77
I194	S	0.30	-0.15	0.48
I194	V	0.37	0.78	1.03
I194	W	0.04	-0.09	1.12
I194	Y	-0.32	-0.01	1.01
F196	A	-0.13	-0.13	-0.02
F196	C	1.74	1.18	0.70
F196	F	1.00	1.00	1.00
F196	G	1.59	-0.30	0.60
F196	H	1.77	-0.24	0.23
F196	I	1.32	1.12	0.81
F196	K	-0.13	-0.13	-0.02
F196	L	1.77	1.17	1.09
F196	M	1.65	0.71	0.93
F196	N	-0.13	-0.13	-0.02
F196	P	0.05	0.39	0.42
F196	Q	1.00	-0.25	0.40
F196	R	-0.13	-0.13	-0.02
F196	S	1.58	-1.57	0.29
F196	V	1.40	0.68	0.51
F196	W	1.01	0.38	0.88
F196	Y	1.41	0.97	0.73

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EXAMPLE 11

Cloning and Expression of a *Sinorhizobium meliloti* RSM02162 *M. smegmatis* Perhydrolase Homologue

In this Example, cloning and expression of a *S. meliloti* perhydrolase homologue are described. The sequences used in cloning and expression are provided below. The gene RSM02162 (SEQ ID NO:625) was synthesized by DNA2.0. The gene was given the designation "G00355" and was provided cloned into the commercially available vector, pDRIVE (InvivoGen). The gene was amplified by PCR from this clone using the primer set G00355rbsF/ G00355R, *Taq* DNA polymerase (Roche) as per the manufacturer's directions, with G00355 as the template (10 ng/50 µl reaction) and 10 picomoles (per 50 µl reaction) of each primer. The amplification was carried out in an MJ Research PCR machine using 30 cycles of (1 minute at 95°C; 1 minute at 55°C; and 1 minute at 72°C). The amplification of the correct size fragment was confirmed by agarose gel electrophoresis. The fragment was cloned directly into pCR2.1TOPO (Invitrogen) and transformed into *E. coli* Top10 cells (Invitrogen). Transformants were selected on L agar containing carbenicillin (100 µg/ml) at 37°C. The correct construct was confirmed by sequence analysis and designated "pMC355rbs." Figure 20 provides a map of this plasmid.

Primer sequences:
G00355rbsF
5'-ggccctaacaggaggaattaaccatggtggaaaaacgttccgttctgtgc-3' (SEQ ID NO:626)

G00355R
5'-Gcgcgcttagaacagagccgctactttgtcagc-3' (SEQ ID NO:627)

Gene sequence (including stop codon) of RSM02162:

5'-

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atggtggaaaaacgttccgttctgtgctttggtgattctctgacttggggctggattccggtgaaagagagctccccaactctgcgtt
acccatacgaacagcgttggaccggtgctatggctgcacgtctgggtgatggttaccacatcattgaagaaggcctgtccgctcgt
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aacatccgcctgggtcatgctattgctgacaaagtagcggctctgttctaa-3’ (SEQ ID NO:625)

G00355 Protein sequence:

MVEKRSVLCFGDSLTWGWIPVKESSPTLRYPYEQRWTGAMAARLGDGYHIIEEG
LSARTTSLDDPNDARLNGSTYLPMALASHLPLDLVIIMLGTNDTKSYFHRTPYEIA
NGMGKLVGQVLTCAGGVGTPYPAPKVLVVAPPPLAPMPDPWFEGMFGGGYEKS
KELSGLYKALADFMKVEFFAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF
(SEQ ID NO:628)

Complete sequence of pDRIVEG00355:

gcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagc
gggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttg
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aggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcc
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cggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccg
cctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaaga
(SEQ ID NO:629)

Complete sequence pMC355rbs:

GC821-2

agcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaag
cgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgtt
gtgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcttggtaccgagctcggatcca
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gctatggctgcacgtctgggtgatggttaccacatcattgaagaaggcctgtccgctcgtactactagcctggacgacccaaacga
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ccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaac
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cctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatg
gaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcg
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gagcgcagataccaaatactgttcttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcg
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aggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacct
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ccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgca
gccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaag (SEQ ID NO:630)

Expression of the Homologue from pMC355rbs

To express the *S. meliloti* RSM02162 protein from the plasmid pMC355rbs (*See*, Figure 20, for a map of this plasmid), a single colony was inoculated into a 5 mls of L broth containing 100 μg/ml carbenicillin and grown overnight at 37°C with shaking at 200 rpm. Lysates were prepared by pelleting the cells from 1 ml of the overnight culture by centrifugation and lysed with BugBuster (Novagen). The supernatants were assayed using the pNA activity assay, perhydrolysis assay, and a pNC6 assay (to test its ability to hydrolyze carbon chains longer than C4), as described herein.

Assay Results

The following Table (Table 11-1) provides a comparison of the hydrolysis activity of pNA by G00355 as compared to the *M. smegmatis* perhydrolase

Table 11-1. pNA Hydrolysis Activity

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Strain	pNA Hydrolysis Rate*	Rate Compared to Perhydrolase
E. coli/pMSATNcoI	85	1
E. coli/pMC355rbs	80	0.94
E. coli/pCR2.1	34.6	0.41

*Rate is absorbance units/min read at 405 nm in a spectrophotometer.

The following Table (Table 11-2) provides a comparison of the perhydrolysis of triacetin by G00355 compared to the *M. smegmatis* perhydrolase.

Table 11-2. Triacetin Perhydrolysis Activity

Strain	Perhydrolysis Activity	
	Max	Vmax
E. coli/pMSATNcoI	1.04	11.88
E. coli/pMC355rbs	1.17	25.05
E. coli/pCR2.1	0.1	2.9

The following Table (Table 11-3) provides a comparison of pNC6 hydrolysis by G00355 compared to the *M. smegmatis* perhydrolase.

Table 11-3. pNC6 Hydrolysis Activity

Strain	pNC6 Hydrolysis Rate*	Rate Compared to Ms. Perhydrolase
E. coli/pMSATNcoI	0.58	1
E. coli/pMC355rbs	6.57	11.3
E. coli/pCR2.1	0.47	0.8

*Rate is absorbance units/min read at 405 nm in a spectrophotometer.

As these results indicate, the homologue RSM02162 from *S. meliloti* identified by amino acid sequence homology to the *M. smegmatis* perhydrolase demonstrated similar, albeit less perhydrolysis activity than the *M. smegmatis* perhydrolase. However, this enzyme exhibited different substrate specificity, as it was able to hydrolyze pNC6, while the wild-type *M. smegmatis* perhydrolase cannot.

The results of the pNC6 hydrolysis assay indicated that certain positions/substitutions provided an improvement in the ability of the enzyme to utilize longer chain substrates The positions and substitutions identified in preliminary screens are provided in the following Table. It is not intended that the present invention be limited to these specific positions and substitutions, as it is contemplated that additional positions and/or substitutions will also provide improved activity on longer chain substrates.

Table 11-4. Positions/Substitutions with Improved Activity in PNC6 Assay

Wild-Type Residue/Position	Amino Acid Variant(s)
L12	G, P, Q
S54	L, T
I153	F, P
F154	Q, S, T, V
I194	G
F196	A, C, G, I, N, P, Q, S, V

EXAMPLE 12

Amplification of Genes Encoding *M. smegmatis* Perhydrolase Homologues from Environmental Isolates

GC821-2

In this Example, methods used to amplify genes encoding *M. smegmatis* perhydrolase homologues from environmental isolates are described.

Organisms from soil samples that were positive for the transesterification reaction were purified to single colonies. To amplify the genes by PCR, the degenerate primer sets 1AF/5AR and 1eF/5iR were used in a PCR reaction containing isolated chromosomal DNA from 8 environmental strains exhibiting the transesterification reaction. The PCR reaction was carried out using *Taq* DNA polymerase (Roche) as per the manufacturer's protocol, with 1 µg of chromosomal DNA added as template and 10 picomoles of each primer in a 50µl reaction. The reaction was carried out for 30 cycles of (1 minute at 95°C; 1 minute at 50°C, and 1 minute at 72°C). Since the partial coding sequence of the perhydrolase gene from *Mycobacterium parafortuitum* was already isolated, the same strain was used as a positive control. The strains were designated as: 2G, 2D, 9B, 14B, 18D, 19C, 20A. As indicated below, 20A was typed as *Mycobacterium parafortuitum*, and 9B is *Mycobacterium gilvum*. Based on protein homology, it was inferred that 2D is also *M. parafortuitum* and 14B is *M. gilvum.*

Primer Sequences

1AF:
5'-gccaagcgaattctgtgtttcggngaytcnyt-3' (SEQ ID NO:631)

5AR:
5'-cgattgttcgcctcgtgtgaartgnrtnccrtc-3' (SEQ ID NO:632)

1eF:
5'-acggtcctgtgctttggngaytcnyt-3' (SEQ ID NO:633)

5iR:
5'-ccgctggtcctcatctggrtgntcnccrtc-3' (SEQ ID NO:634)

Amplification with the above primer sets was expected to yield bands of approximately 500 bp. In all cases except 2G, the 1AF/5AR primer set produced a band of the expected size. In the case of 19C, both primer sets produced bands of the expected size. The ~500 bp bands were purified from agarose gels using a gel purification kit (Qiagen) and analyzed by sequencing. While the strains 2G and 19C yielded bands of the expected size with both primer sets they were not the fragments encoding the *M. smegmatis* perhydrolase homologue.

Partial Sequences of 2D Perhydrolase Homologue and Protein:

Gene:
5'- attctgtgtttcggggattccttgacgtggggatggatccctgtcgaagaaggtgtgcccaccgagcggttcccgcgtga
cgtccggtggaccggcgtgctggccgacctgctgggcgaccgctacgaggtgatcgaggaaggcctgtcggcgcgcacca
ccaccgccgacgacccggccgacccccggctcaacggttcgcagtatctgccgtcgtgtctggccagccatctgccgctg
gacctggtgatcctgatgctcggcatcaacgacaccaaggcgaattttggccgcaccccgttcgacatcgccaccggtat
gggagtgcttgccacgcaggtgctcaccagcgccggtggcgtggggaccagctatcccgcgccgcaggtgctgatcgtgg
cgccgccgccgctgggcgagctgccccacccctggttcgacctggtgttctccggcggccgtgagaagaccgccgagttg
gcccgcgtgtacagcgcgctggcgtcgttcatgaaggtgccgttcttcgacgccggctcggtgatcagcaccgacggcgt
ggacggcacccacttcacacgaggcgaaacaatcga (SEQ ID NO:635)

Protein:
ILCFGDSLTWGWIPVEEGVPTERFPRDVRWTGVLADLLGDRYEVIEEGLSARTTT
ADDPADPRLNGSQYLPSCLASHLPLDLVILMLGINDTKANFGRTPFDIATGMGVL
ATQVLTSAGGVGTSYPAPQVLIVAPPPLGELPHPWFDLVFSGGREKTAELARVYS
ALASFMKVPFFDAGSVISTDGVDGTHFTRGETI (SEQ ID NO:636)

Partial Sequences of 9B Perhydrolase Homologue and Protein:

Gene:
5'-taccgtcgatgtgtggcctcgtgtgaagtgggtgccgttgccaagcgaattctgtgtttcggggattcgttgacgtgggg
ctggatcccggtcgaggaaggtgtacccacccaacgttttccgaagcgggtgcgctggaccggggtgctggccgacgaac
tgggtgctggctatgaggttgtcgaggaggggttgagcgcgcgcaccaccaccgctgacgaccctaccgatccccggctg
aacggctcggactacctccccgcatgcctggccagccacctgccgctggacctggtgatcctgatgctcgggaccaacga
caccaaggcgaatctgaatcgcacacccgtcgacatcgccagcggaatgggcgtcctggccacccaggtgctcaccagcg
cgggcggggtcggcaccagctacccggccccgcaggtgttgatcgtggcaccgccgccgctggccgagatgccgcacccg
tggttcgagctggtcttcgacggcggccgggagaagaccgcccaactggcccgggtgtacagcgcgctggcgtcgttcat
gaaggtgccgttcttcgacgccggatcggtgatcagcaccgacggtgtcgacggcacccacttcacacgaggcgaaacaa
tcgaccgg (SEQ ID NO:637)

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Protein:

GGRCVASCEVGAVAKRILCFGDSLTWGWIPVEEGVPTQRFPKRVRWTGVLADEL
GAGYEVVEEGLSARTTTADDPTDPRLNGSDYLPACLASHLPLDLVILMLGTNDTK
ANLNRTPVDIASGMGVLATQVLTSAGGVGTSYPAPQVLIVAPPLAEMPHPWFEL
VFDGGREKTAQLARVYSALASFMKVPFFDAGSVISTDGVDGTHFTRGETIDR
(SEQ ID NO:638)

Partial Sequences of 14B Perhydrolase Homologue and Protein:

Gene:
5'- attctgtgtttcggagattcgttgacgtggggctggatcccggtcgaggaaggtgtacccacccaacgttttccgaagcg
ggtgcgctggaccggggtgctggccgacgaactgggtgctggctatgaggttgtcgaggaggggttgagcgcgcgcacca
ccaccgctgacgaccctaccgatccccggctgaacggctcggactacctccccgcatgcctggccagccacctgccgctg
gacctggtgatcctgatgctcgggaccaacgacaccaaggcgaatctgaatcgcacacccgtcgacatcgccagcggaat
gggcgtcctggccacccaggtgctcaccagcgcgggcggggtcggcaccagctacccggccccgcaggtgttgatcgtgg
caccgccgccgctggccgagatgccgcacccgtggttcgagctggtcttcgacggcggccgggagaagaccgcccaactg
gcccgggtgtacagcgcgctggcgtcgttcatgaaggtgccgttcttcgacgccggatcggtgatcagcaccgacggtgt
cgacggcacccacttcacacgagg (SEQ ID NO:639)

Protein:
ILCFGDSLTWGWIPVEEGVPTQRFPKRVRWTGVLADELGAGYEVVEEGLSARTT
TADDPTDPRLNGSDYLPACLASHLPLDLVILMLGTNDTKANLNRTPVDIASGMGV
LATQVLTSAGGVGTSYPAPQVLIVAPPLAEMPHPWFELVFDGGREKTAQLARV
YSALASFMKVPFFDAGSVISTDGVDGTHFTR (SEQ ID NO:640)

Partial Sequences of 20A Perhydrolase Homologue and Protein:

Gene:
5'- ttgccaagcggaattctgtgtttcggggattctttgacgtggggatggatccctgtcgaagaaggtgtgcccaccgagcg
gttcccgcgtgacgtccggtggaccggcgtgctggccgacctgctgggcgaccgctacgaggtgatcgaggaaggcctgt
cggcgcgcaccaccaccgccgacgacccggccgacccccggctcaacggttcgcagtatctgccgtcgtgtctggccagc
catctgccgctggacctggtgatcctgatgctcggcatcaacgacaccaaggcgaattttggccgcaccccgttcgacat
cgccaccggtatgggagtgcttgccacgcaggtgctcaccagcgccggtggcgtggggaccagctatcccgcgccgcagg
tgctgatcgtggcgccgccgccgctgggcgagctgccccacccctggttcgacctggtgttctccggcggccgtgagaag
accgccgagttggcccgcgtgtacagcgcgctggcgtcgttcatgaaggtgccgttcttcgacgccggctcggtgatcag
caccgacggcgtggacggcacccacttcacacgaggcgaaacaatcga-3' (SEQ ID NO:641)

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Protein:
LPSGILCFGDSLTWGWIPVEEGVPTERFPRDVRWTGVLADLLGDRYEVIEEGLSA
RTTTADDPADPRLNGSQYLPSCLASHLPLDLVILMLGINDTKANFGRTPFDIATGM
GVLATQVLTSAGGVGTSYPAPQVLIVAPPPLGELPHPWFDLVFSGGREKTAELAR
VYSALASFMKVPFFDAGSVISTDGVDGTHFTRGETI (SEQ ID NO:642)

Identification of the Natural Isolates

To type the environmental isolates used in this Example, plates of the purified strains were sent to MIDI for 16S rRNA typing. 20A is *Mycobacterium parafortuitum*, 9B is *Mycobacterium gilvum*. By protein homology we infer that 2D is also *M. parafortuitum* and 14B is *M. gilvum*.

EXAMPLE 13

Sequence and Taxonomic Analyses of Perhydrolase Homologues

In this Example, sequence and taxonomic analyses of *M. smegmatis* perhydrolase homologues are provided

Taxonomic Assignment

The basic "List of 60" protein sequences accessed from public databases and used for construction of primer sets for screening of metagenomic libraries (BRAIN) was converted into a document illustrating the microbial taxonomic origins of the proteins, as described below. This information was used to produce the following alignment.

```
                              1                                                50
                 MSAT    (1) -------------MAKRILCFGDSLUWGWVPVEDGAPU-ERFAPDVRWUG
   14B  natural isolate   (1) -----------------ILCFGDSLTWGWIPVEEGVPT-QRFPKRVRWTG
                  20A    (1) -------------LPSGILCFGDSLTWGWIPVEEGVPT-ERFPRDVRWTG
    2D  natural isolate   (1) -----------------ILCFGDSLTWGWIPVEEGVPT-ERFPRDVRWTG
     9B Natural Isolate   (1) -GGRCVASCEVGAVAKRILCFGDSLTWGWIPVEEGVPT-QRFPKRVRWTG
   M. parafortuitum CO1   (1) -------------MAKRILCFGDSLTWGWIPVEEGVPT-ERFPRDVRWTG
            Sm-RSM05666   (1) --------------MKTVLCYGDSLTWGYDATGSG-----RHALEDRWPS
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```
                     At-Q8UAC0  (1) --------------MKTVLAFGDSLTWGADPATG---L--RHPVEHRWPD
                     At-Q8UFG4  (1) -------------MVKSVLCFGDSLTWGSNAETGG-----RHSHDDLWPS
                   M091_M4aE11  (1) --------------MKTILAYGDSLTYGANPIPGGP----RHAYEDRWPT
                  Ml-RMLO00301  (1) MAGGTRLDECTGERMKTVLCYGDSLTWGYNAEGG------RHALEDRWPS
         P.dejongeii RVM04532  (1) --------------MKTILCFGDSNTWGYDPASMTAPFPRRHGPEVRWTG
 Q92XZ1 Sinorhizobium meliloti  (1) ---------MEETVARTVLCFGDSNTHGQVPGRGPLDR---YRREQRWGG
    Q98MY5 Mesorhizobium loti  (1) --------------MKTVLCYGDSLTWGYNAEGG------RHALEDRWPS
                   RSM02162_Sm  (1) -----------MVEKRSVLCFGDSLTWGWIPVKESSPT-LRYPYEQRWTG
                   S261_M2aA12  (1) --------------MKNILAFGDSLTWGFVAGQDAR-----HPFETRWPN
Sma1993 Sinorhizobium meliloti  (1) MTINSHSWRTLMVEKRSVLCFGDSLTWGWIPVKESSPT-LRYPYEQRWTG
                     Consensus  (1)                KTILCFGDSLTWGWIPV EG P   RHP E RW G

                                    51                                             100
                          MSAT (37) VLAQQLGADFEVIE--EGLSARUUNIDDPUDPRL-NGASYLPSCLAUHLP
           14B  natural isolate (33) VLADELGAGYEVVE--EGLSARTTTADDPTDPRL-NGSDYLPACLASHLP
                           20A (37) VLADLLGDRYEVIE--EGLSARTTTADDPADPRL-NGSQYLPSCLASHLP
            2D  natural isolate (33) VLADLLGDRYEVIE--EGLSARTTTADDPADPRL-NGSQYLPSCLASHLP
            9B Natural Isolate (49) VLADELGAGYEVVE--EGLSARTTTADDPTDPRL-NGSDYLPACLASHLP
          M. parafortuitum CO1 (37) VLADLLGDRYEVIE--EGLSARTTTAEDPADPRL-NGSQYLPSCLASHLP
                    Sm-RSM05666 (32) VLQKALGSDAHVIA--EGLNGRTTAYDDHLADCDRNGARVLPTVLHTHAP
                     At-Q8UAC0 (32) VLEAELAGKAKVHP--EGLGGRTTCYDDHAGPACRNGARALEVALSCHMP
                     At-Q8UFG4 (33) VLQKALGSDVHVIFTHEGLGGRTTAYDDHTGDCDRNGARLLPTLLHSHAP
                   M091_M4aE11 (33) ALEQGLGGKARVIA--EGLGGRTTVHDDWFANADRNGARVLPTLLESHSP
                  Ml-RMLO00301 (45) VLQASLGGGVQVIA--DGLNGRTTAFDDHLAGADRNGARLLPTALTTHAP
         P.dejongeii RVM04532 (37) VLAKALGAGFRVIE--EGQNGRTTVHEDPLNICR-KGKDYLPACLESHKP
 Q92XZ1 Sinorhizobium meliloti (39) VLQGLLGPNWQVIE--EGLSGRTTVHDDPIEGSLKNGRIYLRPCLQSHAP
    Q98MY5 Mesorhizobium loti (31) VLQASLGGGVQVIA--DGLNGRTTAFDDHLAGADRNGARLLPTALTTHAP
                   RSM02162_Sm (39) AMAARLGDGYHIIE--EGLSARTTSLDDPNDARL-NGSTYLPMALASHLP
                   S261_M2aA12 (32) ALAAGLGGKARVIE--EGQNGRTTVFDDAATFESRNGSVALPLLLISHQP
Sma1993 Sinorhizobium meliloti (50) AMAARLGDGYHIIE--EGLSARTTSLDDPNDARL-NGSTYLPMALASHLP
                     Consensus (51) VLA  LGG Y VIE  EGLSGRTT  DDP D  L NGS YLPT LASHLP

                                    101                                            150
                          MSAT (84) LDLVIIMLGUNDUKAYFRRUPLDIA--LGMSVLVUQVLUSAGGVGUUYPA
           14B  natural isolate (80) LDLVILMLGTNDTKANLNRTPVDIA--SGMGVLATQVLTSAGGVGTSYPA
                           20A (84) LDLVILMLGINDTKANFGRTPFDIA--TGMGVLATQVLTSAGGVGTSYPA
            2D  natural isolate (80) LDLVILMLGINDTKANFGRTPFDIA--TGMGVLATQVLTSAGGVGTSYPA
            9B Natural Isolate (96) LDLVILMLGTNDTKANLNRTPVDIA--SGMGVLATQVLTSAGGVGTSYPA
          M. parafortuitum CO1 (84) LDLVILMLGTNDTKANFGRTPFDIA--TGMGVLATQVLTSAGGVGTSYPA
                    Sm-RSM05666 (80) LDLIVFMLGSNDMKPIIHGTAFGAV--KGIERLVNLVRRHDWPTETE-EG
                     At-Q8UAC0 (80) LDLVIIMLGTNDIKPVHGGRAEAAV--SGMRRLAQIVETFIYKPREA--V
                     At-Q8UFG4 (83) LDMVIIMLGTNDMKPAIHGSAIVAFTMKGVERLVKLTRNHVWQVSDW-EA
                   M091_M4aE11 (81) LDLIVIMLGTNDIKPHHGRTAGEAG--RGMARLVQIIRGHYAGRMQD--E
                  Ml-RMLO00301 (93) IDLIVIMLGANDMKPWIHGNPVAAK--QGIQRLIDIVRGHDYPFDWP--A
         P.dejongeii RVM04532 (84) LDLVILMLGTNDLKSTFNVPPGEIA--AGAGVLGRMILAGDAGPENR--P
 Q92XZ1 Sinorhizobium meliloti (87) LDLIIIMLGTNDLKRRFNMPPSEVA--MGIGCLVHDIRELSPGRTGN--D
    Q98MY5 Mesorhizobium loti (79) IDLIVIMLGANDMKPWIHGNPVAAK--QGIQRLIDIVRGHDYPFDWP--A
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                   RSM02162_Sm   (86) LDLVIIMLGTNDTKSYFHRTPYEIA--NGMGKLVGQVLTCAGGVGTPYPA
                   S261_M2aA12   (80) LDLVIIMLGTNDIKFAARCRAFDAS--MGMERLIQIVRSANYMKGYK--I
Sma1993 Sinorhizobium meliloti   (97) LDLVIIMLGTNDTKSYFHRTPYEIA--NGMGKLVGQVLTCAGGVGTPYPA
                     Consensus  (101) LDLVIIMLGTNDMKA   RTP DIA   GMGRLV  VLT AGGVG    A

                                      151                                            200
                          MSAT  (132) PKVLVVSPPPLAPM-PHPWFQLIF-EGGEQKUUELARVYSALASFMKVPF
          14B  natural isolate  (128) PQVLIVAPPPLAEM-PHPWFELVF-DGGREKTAQLARVYSALASFMKVPF
                           20A  (132) PQVLIVAPPPLGEL-PHPWFDLVF-SGGREKTAELARVYSALASFMKVPF
           2D  natural isolate  (128) PQVLIVAPPPLGEL-PHPWFDLVF-SGGREKTAELARVYSALASFMKVPF
            9B Natural Isolate  (144) PQVLIVAPPPLAEM-PHPWFELVF-DGGREKTAQLARVYSALASFMKVPF
          M. parafortuitum C01  (132) PQVLIVAPPPLGEL-PHPWFDLVF-SGGREKTAELARVYSALASFMKVPF
                   Sm-RSM05666  (127) PEILIVSPPPLCET--ANSAFAAMFAGGVEQSAMLAPLYRDLADELDCGF
                     At-Q8UAC0  (126) PKLLIVAPPPCVAG---PGGEPAG-GRDIEQSMRLAPLYRKLAAELGHHF
                     At-Q8UFG4  (132) PDVLIVAPPQLCETANPFMGAIFRDAIDESAMLASVFTYRDLADELDCGF
                   M091_M4aE11  (127) PQIILVSPPPIILGDWADMMDHFGPHEAIATSVDFAREYKKRADEQKVHF
                  Ml-RML000301  (139) PQILIVSPPVVSRT--ENADFREMFAGGDEASKQLAPQYAALADEVGCGF
          P.dejongeii RVM04532  (130) PQLLLMCPPKVRDLSAMPDLDAKI-PHGAARSAEFPRHYKAQAVALKCEY
 Q92XZ1 Sinorhizobium meliloti  (133) PEIMIVAPPPMLED--LKEWESIF-SGAQEKSRKLALEFEIMADSLEAHF
     Q98MY5 Mesorhizobium loti  (125) PQILIVSPPVVSRT--ENADFREMFAGGDEASKQLAPQYAALADEVGCGF
                   RSM02162_Sm  (134) PKVLVVAPPPLAPM-PDPWFEGMF-GGGYEKSKELSGLYKALADFMKVEF
                   S261_M2aA12  (126) PEILIISPPSLVPT--QDEWFNDLWGHAIAESKLFAKHYKRVAEELKVHF
Sma1993 Sinorhizobium meliloti  (145) PKVLVVAPPPLAPM-PDPWFEGMF-GGGYEKSKELSGLYKALADFMKVEF
                     Consensus  (151) PQVLIVAPPPL EM   P FE VF  GG EKS  LARVY ALAD MKV F

                                      201                                    241
                          MSAT  (180) FDAGSVISUDGVDGIHFUEANNRDLGVALAEQVRSLL----(SEQ ID NO:643)
          14B  natural isolate  (176) FDAGSVISTDGVDGTHFTR----------------------(SEQ ID NO:644)
                           20A  (180) FDAGSVISTDGVDGTHFTRGETI------------------(SEQ ID NO:645)
           2D  natural isolate  (176) FDAGSVISTDGVDGTHFTRGETI------------------(SEQ ID NO:646)
            9B Natural Isolate  (192) FDAGSVISTDGVDGTHFTRGETIDR----------------(SEQ ID NO:647)
          M. parafortuitum C01  (180) FDAGSVISTDGVDGIHFTRGEQST-----------------(SEQ ID NO:648)
                   Sm-RSM05666  (175) FDGGSVARTTPIDGVHLDAENTRAVGRGLEPVVRMMLGL--(SEQ ID NO:649)
                     At-Q8UAC0  (172) FDAGSVASASPVDGVHLDASATAAIGRALAAPVRDILG---(SEQ ID NO:650)
                     At-Q8UFG4  (182) FDAGSVARTTPVDGVHLDAENTRAIGRGLEPVVRMMLGL--(SEQ ID NO:651)
                   M091_M4aE11  (177) FDAGTVATTSKADGIHLDPANTRAIGAGLVPLVKQVLGL--(SEQ ID NO:652)
                  Ml-RML000301  (187) FDAGTVAQTTPLDGVHLDAENTRNIGKALTSVVRVML----(SEQ ID NO:653)
          P.dejongeii RVM04532  (179) FNSQEIVETSPVDGIHLEASEHLKLGEALAEKVKVLLG---(SEQ ID NO:654)
 Q92XZ1 Sinorhizobium meliloti  (180) FDAGTVCQCSPADGFHIDEDAHRLLGEALAQEVLAIGWPDA(SEQ ID NO:655)
     Q98MY5 Mesorhizobium loti  (173) FDAGTVAQTTPLDGVHLDAENTRNIGKALTSVVRVMLEL--(SEQ ID NO:656)
                   RSM02162_Sm  (182) FAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF----(SEQ ID NO:657)
                   S261_M2aA12  (174) FDAGTVAVADKTDGGHLDAVNTKAIGVALVPVVKSILAL--(SEQ ID NO:658)
Sma1993 Sinorhizobium meliloti  (193) FAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF----(SEQ ID NO:659)
                     Consensus  (201) FDAGSVISTD VDGIHLDA  T  IG AL   VR LL    (SEQ ID NO:660)
```

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The alignment tree from the CLUSTALW alignment (which approximates to a phylogenetic tree) suggests 3 or 4 groupings.

From this alignment, a hypothetical protein sequence was constructed from the consensus sequence. Where no consensus existed the site was filled with the Per amino acid; gaps were ignored. This provided a Per-consensus sequence:

```
1    TILCFGDSLT WGWIPVEEGA PTERHPPEVR WTGVLAQQLG GDYEVIEEGL
51   SGRTTNIDDP TDPRLNGSSY LPTCLASHLP LDLVIIMLGT NDMKAYFRRT
101  PLDIALGMGR LVTQVLTSAG GVGTTYPAPQ VLIVAPPPLA EMPHPWFELV
151  FEGGEEKSTE LARVYSALAD FMKVPFFDAG SVISTDGVDG IHLDAANTRD
201  IGVALAEQVR SLL  (SEQ ID NO:661)
```

This consensus sequence was used for a BLASTP search against a non-redundant database. This search identified 55 hits. The majority of the 'hits' were GDSL or GDSI type molecules covering a wide range of microbial diversity. However, only the first 14 'hits' had e-values and bit-values in the reliable range. At first sight, this appeared to provide further molecules with a GDSL/N – G/ARTT motif, but this was found to be due to differences in coding (Swiss Prot vs GenBank)

The screening of 3 environmental libraries (at BRAIN) resulted in 10 clones with a GDSL motif. A further 2 clones were derived from the BRAIN library. The following Table (Table 13-1) lists the clones and indicates their activity.

Table 13-1. Clones with GDSL Motifs

Library	Clone	Perhydrolase Activity
S248Fa	S248_M40cD4	No
S248Fa	S248_M44aA5	No
S248Fa	S248_M18bH12	Not Perhydrolase
S248Fa	S248_M36bC5	Not Perhydrolase

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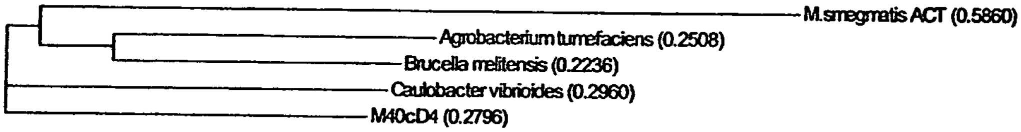
S248Fa	S248_M50cD9	Not Perhydrolase
S248Fa	S248_M2bB11	? Low
S261	S261_M2aA12	Yes
S279	S279_M75bA2	Not done
S279	S279_M11aC12	Not GDSL
S279	S279_M70aE8	? Low
M091	M091_M4aE11	Not tested
BRAIN	Est114	No
BRAIN	Est105	Not done

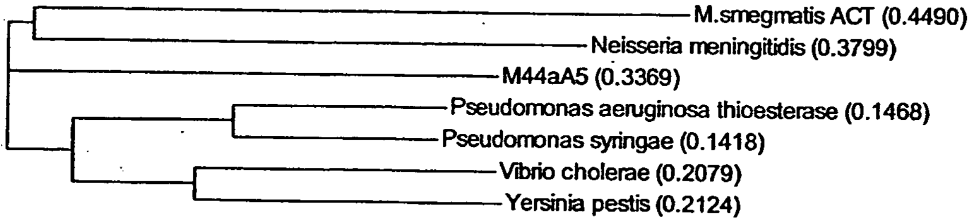
M40cD4

Strongest hit: arylesterase of *Brucella melitensis* (46% identical). Motifs: GDSL – GAND; GQTT instead of GRTT. Sequence alignment against the core list of organisms places it close to *Caulobacter vibrioides* and *Brucella melitensis* in the alpha-*Proteobacteria*.



M44aA5

Strongest hit:Acyl-CoA thioesterase of *Pseudomonas aeruginosa* (43% identical). Motifs: GDSL – GGND; no GRTT or equivalent. Sequence alignment against the core list of organisms places it close to *Pseudomonas* sp in the gamma-*Proteobacteria.*



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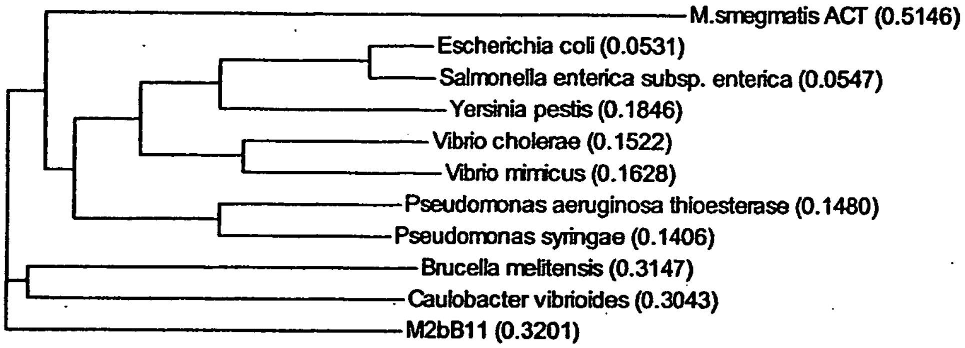
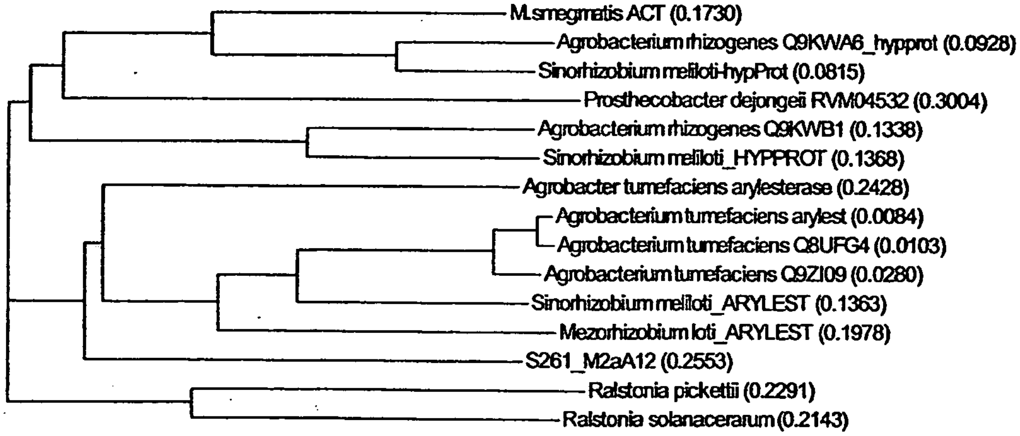
M2bB11

Strongest hit: arylesterase of *Brucella melitensis*. Motifs: GDSL – GAND; no GRTT or equivalent. Sequence alignment against the core list of organisms shows no strong association placing it between the alpha- and gamma-*Proteobacteria*.



M2aA12

Strongest hit: arylesterase of *Agrobacterium tumefaciens* (42% identical) Motifs: GDSL – GRTT – GTND. Sequence alignment against the core list of organisms places it close to *Agrobacterium tumefaciens* in the alpha-*Proteobacteria*.



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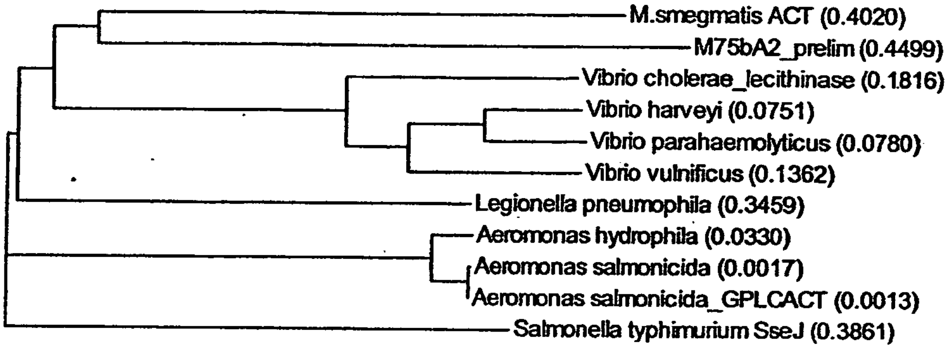
M75bA2

Strongest hit: incomplete. BLAST search revealed nothing significant. Motifs: GDSL – GTND; no GRTT or equivalent. Sequence alignment against the core list of organisms shows no convincing associations. The closest neighbors appear to be the *Vibrio – Aeromonas* groups of the gamma-*Proteobacteria.*



M70aE8

Strongest hit: acyl-CoA thioesterase from *E. coli* (30% identical), and aryl esterase hydrolase from *Vibrio mimicus* (27% identical). Based on incomplete sequence GDSL-type esterase (BRAIN) from *Neisseria meningitidis* (50% identical). Motifs: GDSL – GGND; no GRTT – replaced with GRTV. Sequence alignment against the core list of organisms shows the closest association to *Neisseria meningitidis*, a member of the beta-Proteobacteria.

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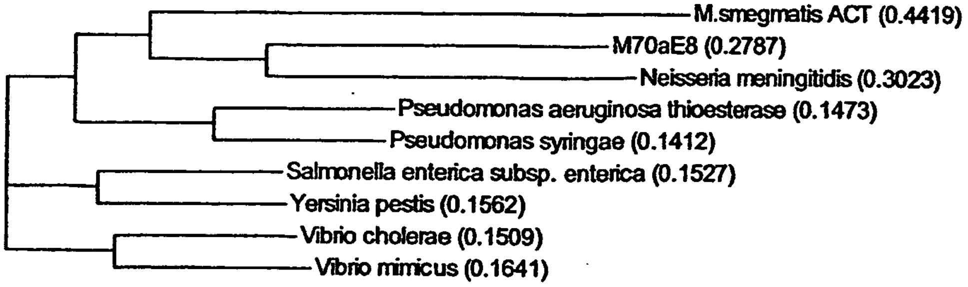
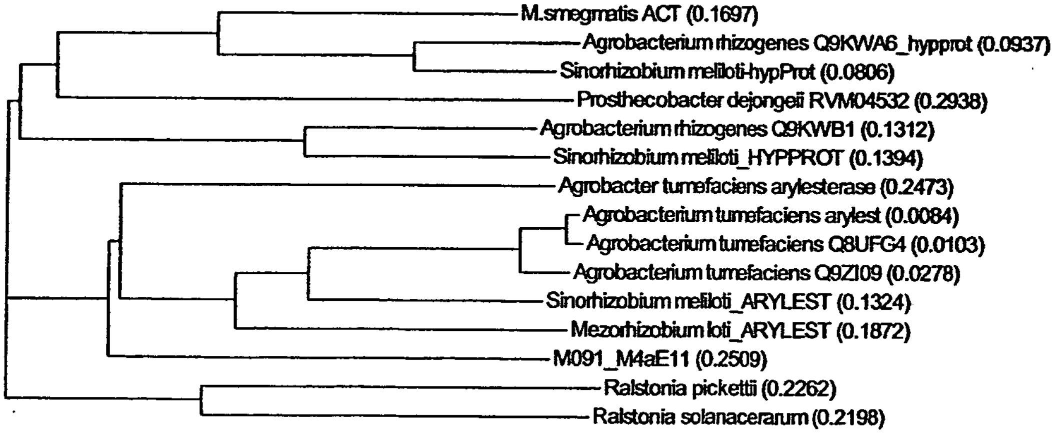


M4aE11

Strongest hit: arylesterase from *Agrobacterium tumefaciens* (59% identity) Motifs: GDSL – GRTT – GTND. Sequence alignment against the core list of organisms shows the closest association to members of the alpha-*Proteobacteria* such as *Agrobacterium.*



Est114

Strongest hit: phosphatidylcholine sterol acyltransferase from *Aeromonas hydrophila* (gamma-*Proteobacteria*) (30% identical). Motifs: GDSL – GPND; no GRTT

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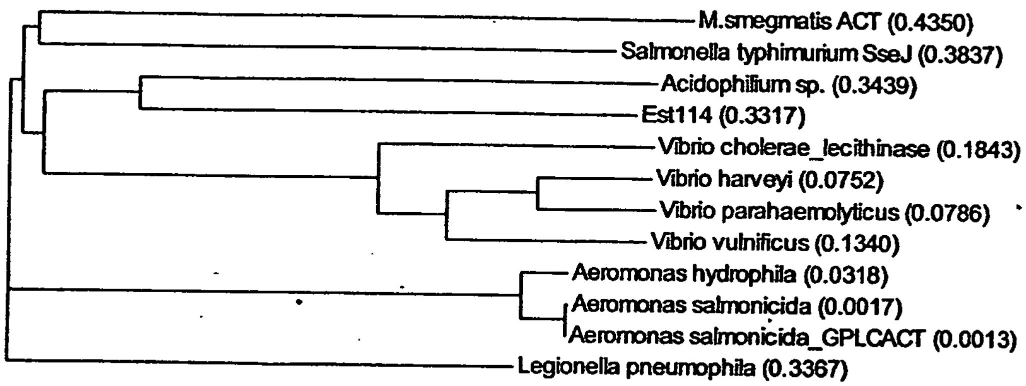
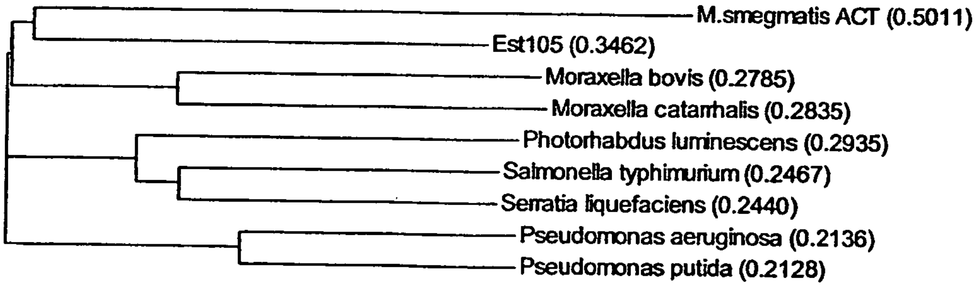
but GATT may be an equivalent. Sequence alignment against the core list of organisms shows the closest association to *Acidophilium* sp. and *Aeromonas/Vibrio* within the gamma-*Proteobacteria.*



Est105

Strongest hit: *Pseudomonas aeruginosa* outer membrane esterase, and hypothetical protein *Pseudomonas putida* (27% identical). Motifs: GDSL – GAND, no GRTT or equivalent. Sequence alignment against the core list of organisms shows the closest association to members of the gamma-*Proteobacteria.*



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An overall alignment of these clones/sequences (here shown underlined) indicates that they are scattered throughout the alignment tree of strains indicating that the metagenomic screening has provided a variety of sequences and not a limited diversity.

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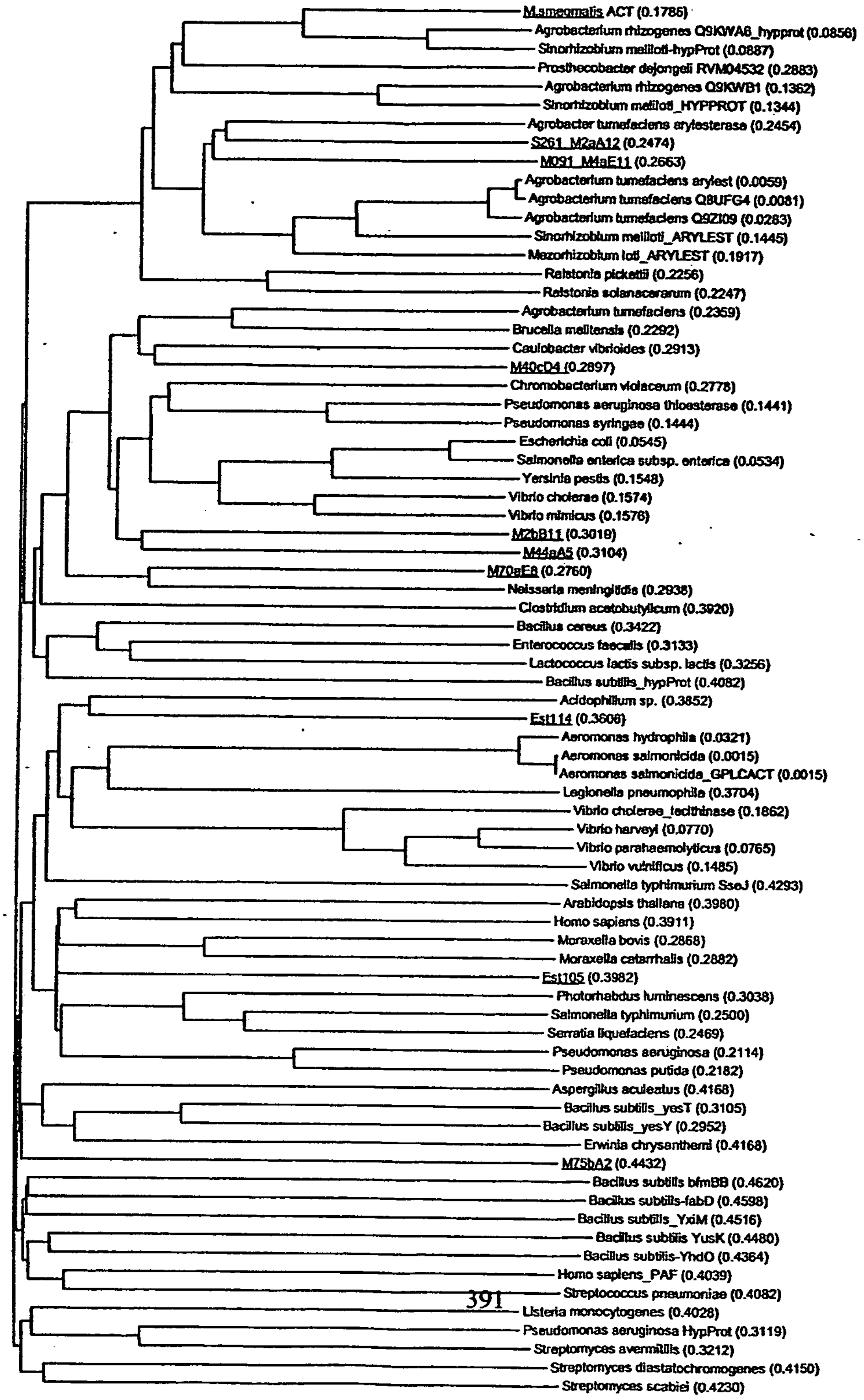
M.smegmatis ACT (0.1786)
Agrobacterium rhizogenes Q9KWA6_hypprot (0.0856)
Sinorhizobium meliloti-hypProt (0.0887)
Prosthecobacter dejongeii RVM04532 (0.2883)
Agrobacterium rhizogenes Q9KWB1 (0.1362)
Sinorhizobium meliloti_HYPPROT (0.1344)
Agrobacter tumefaciens arylesterase (0.2454)
S261_M2aA12 (0.2474)
M091_M4aE11 (0.2663)
Agrobacterium tumefaciens arylest (0.0059)
Agrobacterium tumefaciens Q8UFG4 (0.0081)
Agrobacterium tumefaciens Q9ZI09 (0.0283)
Sinorhizobium meliloti_ARYLEST (0.1445)
Mezorhizobium loti_ARYLEST (0.1917)
Ralstonia pickettii (0.2256)
Ralstonia solanacearum (0.2247)
Agrobacterium tumefaciens (0.2359)
Brucella melitensis (0.2292)
Caulobacter vibrioides (0.2913)
M40cD4 (0.2897)
Chromobacterium violaceum (0.2778)
Pseudomonas aeruginosa thioesterase (0.1441)
Pseudomonas syringae (0.1444)
Escherichia coli (0.0545)
Salmonella enterica subsp. enterica (0.0534)
Yersinia pestis (0.1548)
Vibrio cholerae (0.1574)
Vibrio mimicus (0.1576)
M2bB11 (0.3019)
M44aA5 (0.3104)
M70aE8 (0.2760)
Neisseria meningitidis (0.2938)
Clostridium acetobutylicum (0.3920)
Bacillus cereus (0.3422)
Enterococcus faecalis (0.3133)
Lactococcus lactis subsp. lactis (0.3256)
Bacillus subtilis_hypProt (0.4082)
Acidophilium sp. (0.3852)
Est114 (0.3606)
Aeromonas hydrophila (0.0321)
Aeromonas salmonicida (0.0015)
Aeromonas salmonicida_GPLCACT (0.0015)
Legionella pneumophila (0.3704)
Vibrio cholerae_lecithinase (0.1862)
Vibrio harveyi (0.0770)
Vibrio parahaemolyticus (0.0765)
Vibrio vulnificus (0.1485)
Salmonella typhimurium SseJ (0.4293)
Arabidopsis thaliana (0.3980)
Homo sapiens (0.3911)
Moraxella bovis (0.2868)
Moraxella catarrhalis (0.2882)
Est105 (0.3982)
Photorhabdus luminescens (0.3038)
Salmonella typhimurium (0.2500)
Serratia liquefaciens (0.2469)
Pseudomonas aeruginosa (0.2114)
Pseudomonas putida (0.2182)
Aspergillus aculeatus (0.4168)
Bacillus subtilis_yesT (0.3105)
Bacillus subtilis_yesY (0.2952)
Erwinia chrysanthemi (0.4168)
M75bA2 (0.4432)
Bacillus subtilis bfmBB (0.4620)
Bacillus subtilis-fabD (0.4598)
Bacillus subtilis_YxiM (0.4516)
Bacillus subtilis YusK (0.4480)
Bacillus subtilis-YhdO (0.4364)
Homo sapiens_PAF (0.4039)
Streptococcus pneumoniae (0.4082)
Listeria monocytogenes (0.4028)
Pseudomonas aeruginosa HypProt (0.3119)
Streptomyces avermitilis (0.3212)
Streptomyces diastatochromogenes (0.4150)
Streptomyces scabiei (0.4230)

Gene Mining for GRTT – Type Esterases
(clones with perhydrolase activity)

Sinorhizobium meliloti Sma1993-hypothetical protein_Sme
Motifs: GDSL – ARTT – GTND

Sinorhizobium meliloti Q92XZ1-hypothetical protein_Sme
Motifs: GDSN – GRTT – GTND

Mesorhizobium loti Q98MY5-arylesterase_Mlo
Motifs:GDSL – GRTT – GAND

Moraxella bovis AAK53448 (lipase)
Motifs: GDSL – GSND, no GRTT or equivalent in this sequence order.
(perhydrolase activity low, questionable sequence)

Agrobacterium tumefaciens Q8UACO
Motifs: GDSL – GRTT – GTND

Agrobacterium tumefaciens Q8UFG4
Motifs: GDSL – GRTT – GTND

Mesorhizobium loti RMLO00301
Motifs: GDSL – GRTT – GAND

Sinorhizobium meliloti RSM05666
Motifs: GDSL – GRTT – GSND
(this clone was inactive for perhydrolase activity;
and probably represents a <u>false negative</u>)

Sinorhizobium meliloti RSM02162
Motifs: GDSL – ARTT – GTND

Prosthecobacter dejongeii RVM05432
Motifs: GDSN – GRTT – GTND

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A GDSx_1 –x_2RTT – Gx_3ND motif characterizes the active clones/sequences, where:

X_1 = L or N
X_2 = A or G
X_3 = T or A or S

The *Moraxella bovis* AAK53448 sequence does not fit this pattern and is excluded from the alignment analysis provided below:

Multiple Sequence Alignment of Active Clones/Sequences

```
                                    1                                                  50
                   ACT MSMEG  (1)   -------------MAKRILCFGDSLUWGWVPVEDGAPU-ERFAPDVRWUG
  Q98MY5 Mesorhizobium loti   (1)   --------------MKTVLCYGDSLTWGYNAEGGR------HALEDRWPS
Sma1993 Sinorhizobium meliloti (1)   MTINSHSWRTLMVEKRSVLCFGDSLTWGWIPVKESSPT-LRYPYEQRWTG
 Q92XZ1 Sinorhizobium meliloti (1)   ---------MEETVARTVLCFGDSNTHGQVPGRGPLDR---YRREQRWGG
       P.dejongeii RVM04532  (1)   --------------MKTILCFGDSNTWGYDPASMTAPFPRRHGPEVRWTG
               RSM05666_Sm   (1)   --------------MKTVLCYGDSLTWGYDATGSG-----RHALEDRWPS
               RSM02162_Sm   (1)   -----------MVEKRSVLCFGDSLTWGWIPVKESSPT-LRYPYEQRWTG
                 At-Q8UAC0   (1)   --------------MKTVLAFGDSLTWGADPATGLR-----HPVEHRWPD
                 At-Q8UFG4   (1)   -------------MVKSVLCFGDSLTWGSNAETGG-----RHSHDDLWPS
               M1-RML000301  (1)   MAGGTRLDECTGERMKTVLCYGDSLTWGYNAEGGR------HALEDRWPS
               S261_M2aA12   (1)   --------------MKNILAFGDSLTWGFVAGQDA-----RHPFETRWPN
               M091_M4aE11   (1)   --------------MKTILAYGDSLTYGANPIPGG-PR---HAYEDRWPT
                 Consensus   (1)                 MKTVLCFGDSLTWGY P  G      RHA E RWP

                                    51                                                100
                   ACT MSMEG  (37)  VLAQQLGADFEVIE--EGLSARUUNIDDPUDPRL-NGASYLPSCLAUHLP
  Q98MY5 Mesorhizobium loti   (31)  VLQASLGGGVQVIA--DGLNGRTTAFDDHLAGADRNGARLLPTALTTHAP
Sma1993 Sinorhizobium meliloti (50)  AMAARLGDGYHIIE--EGLSARTTSLDDPNDARL-NGSTYLPMALASHLP
 Q92XZ1 Sinorhizobium meliloti (39)  VLQGLLGPNWQVIE--EGLSGRTTVHDDPIEGSLKNGRIYLRPCLQSHAP
       P.dejongeii RVM04532  (37)  VLAKALGAGFRVIE--EGQNGRTTVHEDPLNICR-KGKDYLPACLESHKP
               RSM05666_Sm   (32)  VLQKALGSDAHVIA--EGLNGRTTAYDDHLADCDRNGARVLPTVLHTHAP
               RSM02162_Sm   (39)  AMAARLGDGYHIIE--EGLSARTTSLDDPNDARL-NGSTYLPMALASHLP
                 At-Q8UAC0   (32)  VLEAELAGKAKVHP--EGLGGRTTCYDDHAGPACRNGARALEVALSCHMP
                 At-Q8UFG4   (33)  VLQKALGSDVHVIFTHEGLGGRTTAYDDHTGDCDRNGARLLPTLLHSHAP
               M1-RML000301  (45)  VLQASLGGGVQVIA--DGLNGRTTAFDDHLAGADRNGARLLPTALTTHAP
               S261_M2aA12   (32)  ALAAGLGGKARVIE--EGQNGRTTVFDDAATFESRNGSVALPLLLISHQP
               M091_M4aE11   (33)  ALEQGLGGKARVIA--EGLGGRTTVHDDWFANADRNGARVLPTLLESHSP
                 Consensus   (51)  VL A LGG   VIE  EGL GRTTAHDD  A   RNGAR LPT L SHAP

                                    101                                               150
                   ACT MSMEG  (84)  LDLVIIMLGUNDUKAYFRRUPLDIA--LGMSVLVUQVLUSAGGVGUUYPA
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```
  Q98MY5 Mesorhizobium loti  (79) IDLIVIMLGANDMKPWIHGNPVAAK--QGIQRLIDIVRGHDYPFDWPAP-
Sma1993 Sinorhizobium meliloti  (97) LDLVIIMLGTNDTKSYFHRTPYEIA--NGMGKLVGQVLTCAGGVGTPYPA
Q92XZ1 Sinorhizobium meliloti  (87) LDLIIIMLGTNDLKRRFNMPPSEVA--MGIGCLVHDIRELSPGRTGN---
       P.dejongeii RVM04532  (84) LDLVILMLGTNDLKSTFNVPPGEIA--AGAGVLGRMILAGDAGPENR-PP
                RSM05666_Sm  (80) LDLIVFMLGSNDMKPIIHGTAFGAV--KGIERLVNLVRRHDWPTETEEG-
                RSM02162_Sm  (86) LDLVIIMLGTNDTKSYFHRTPYEIA--NGMGKLVGQVLTCAGGVGTPYPA
                  At-Q8UAC0  (80) LDLVIIMLGTNDIKPVHGGRAEAAVS--GMRRLAQIVETFIYKPREAVP-
                  At-Q8UFG4  (83) LDMVIIMLGTNDMKPAIHGSAIVAFTMKGVERLVKLTRNHVWQVSDWEAP
               M1-RML000301  (93) IDLIVIMLGANDMKPWIHGNPVAAK--QGIQRLIDIVRGHDYPFDWPAP-
                S261_M2aA12  (80) LDLVIIMLGTNDIKFAARCRAFDAS--MGMERLIQIVRSANYMKGYKIP-
                M091_M4aE11  (81) LDLIVIMLGTNDIKPHHGRTAGEAG--RGMARLVQIIRGHYAGRMQDEP-
                  Consensus (101) LDLVIIMLGTNDMKP  H  P EAA   GM RLV IVR   YG     P

                                     151                                            200
                  ACT MSMEG (132) PKVLVVSPPPLAPMPHPWFQLIFE--GGEQKUUELARVYSALASFMKVPF
  Q98MY5 Mesorhizobium loti (126) -QILIVSPPVVSRTENADFREMFAG--GDEASKQLAPQYAALADEVGCGF
Sma1993 Sinorhizobium meliloti (145) PKVLVVAPPPLAPMPDPWFEGMFG--GGYEKSKELSGLYKALADFMKVEF
Q92XZ1 Sinorhizobium meliloti (132) DPEIMIVAPPPMLEDLKEWESIFS--GAQEKSRKLALEFEIMADSLEAHF
       P.dejongeii RVM04532 (131) QLLLMCPPKVRDLSAMPDLDAKIP--HGAARSAEFPRHYKAQAVALKCEY
                RSM05666_Sm (127) PEILIVSPPPLCETANSAFAAMFAG--GVEQSAMLAPLYRDLADELDCGF
                RSM02162_Sm (134) PKVLVVAPPPLAPMPDPWFEGMFG--GGYEKSKELSGLYKALADFMKVEF
                  At-Q8UAC0 (127) -KLLIVAPPPCVAGPGGEPAGGRD----IEQSMRLAPLYRKLAAELGHHF
                  At-Q8UFG4 (133) -DVLIVAPPQLCETANPFMGAIFRDAIDESAMLASVFTYRDLADELDCGF
               M1-RML000301 (140) -QILIVSPPVVSRTENADFREMFAG--GDEASKQLAPQYAALADEVGCGF
                S261_M2aA12 (127) -EILIISPPSLVPTQDEWFNDLWG--HAIAESKLFAKHYKRVAEELKVHF
                M091_M4aE11 (128) -QIILVSPPPIILGDWADMMDHFGPHEAIATSVDFAREYKKRADEQKVHF
                  Consensus (151)   ILIVSPPPL  T   DF AMFG   G E SK LA  YKALADELK  F

                                     201                                   241
                  ACT MSMEG (180) FDAGSVISUDGVDGIHFUEANNRDLGVALAEQVRSLL---- (SEQ ID NO:662)
  Q98MY5 Mesorhizobium loti (173) FDAGTVAQTTPLDGVHLDAENTRNIGKALTSVVRVMLEL-- (SEQ ID NO:663)
Sma1993 Sinorhizobium meliloti (193) FAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF---- (SEQ ID NO:664)
Q92XZ1 Sinorhizobium meliloti (180) FDAGTVCQCSPADGFHIDEDAHRLLGEALAQEVLAIGWPDA (SEQ ID NO:665)
       P.dejongeii RVM04532 (179) FNSQEIVETSPVDGIHLEASEHLKLGEALAEKVKVLLG--- (SEQ ID NO:666)
                RSM05666_Sm (175) FDGGSVARTTPIDGVHLDAENTRAVGRGLEPVVRMMLGL-- (SEQ ID NO:667)
                RSM02162_Sm (182) FAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF---- (SEQ ID NO:668)
                  At-Q8UAC0 (172) FDAGSVASASPVDGVHLDASATAAIGRALAAPVRDILG--- (SEQ ID NO:669)
                  At-Q8UFG4 (182) FDAGSVARTTPVDGVHLDAENTRAIGRGLEPVVRMMLGL-- (SEQ ID NO:670)
               M1-RML000301 (187) FDAGTVAQTTPLDGVHLDAENTRNIGKALTSVVRVML---- (SEQ ID NO:671)
                S261_M2aA12 (174) FDAGTVAVADKTDGGHLDAVNTKAIGVALVPVVKSILAL-- (SEQ ID NO:672)
                M091_M4aE11 (177) FDAGTVATTSKADGIHLDPANTRAIGAGLVPLVKQVLGL-- (SEQ ID NO:673)
                  Consensus (201) FDAGTVA TSPVDGIHLDAENTR IG ALA VVR LLG    (SEQ ID NO:674)
```

A guide tree (*i.e.*, an approximation of a phylogenetic tree) of the CLUSTALW alignment of active clones/sequences is provided below.

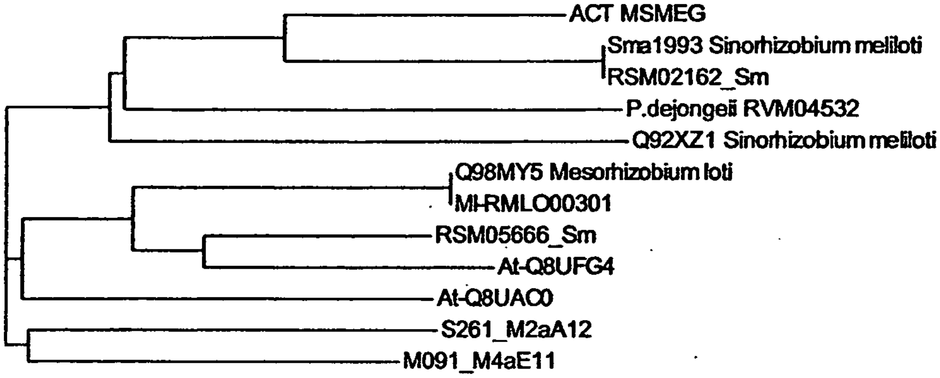

Table 13-2. Similarity and Identity of Clones/Sequences Compared to *M. smegmatis* Perhydrolase

Clone/Sequence	% Identity	% Similarity
Sinorhizobium meliloti Sma1993	55.5	71.6
Sinorhizobium meliloti Q92XZ1	38.7	54.7
Mesorhizobium loti Q98MY5	38.8	53.4
Moraxella bovis AAK53448	5.0	9.7
Agrobacterium tumefaciens Q8UACO	36.7	47.7
Agrobacterium tumefaciens Q8UFG4	37.1	50.4
Mesorhizobium loti RMLO00301	34.8	50.9
Sinorhizobium meliloti RSM05666	37.4	52.5
Sinorhizobium meliloti RSM02162	58.3	75.2

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Prosthecobacter dejongeii RVM05432	41.6	55.7
S261_M2aA12	39.3	54.3
M091_M4aE11	34.7	50.2

Based on the results, the active clones were found to have an overall identity to *M. smegmatis* perhydrolase of 38.7 – 58.3%. *Moraxella bovis* AAK53448 was found to be an exception and the (translated) amino acid sequence is questionable.

Redundancy

From the analyses above, it was evident that some redundancy exists in the alignment provided at the beginning of this Example that will have added undue weighting to the consensus sequence. Also, further GDSL-GRTT sequences were added. Thus, in the revised alignment below, the following changes were made:

Removed:

Natural isolate 14B
Natural isolate 2D
RSM02162_Sm
Q98MY5 Mesorhizobium loti

Added:

BAB16197 (Arh II)
BAB16192 (Arh I)
NP 00197751 (Mlo II)
NP 00216984 (Bce)
NP 522806 (Rso)

Non-redundant alignment:

```
                                  1                                                 50
                    20A     (1) -------------LPSGILCFGDSLTWGWIPVEEGVPTERFP-RDVRWTG
     9B Natural Isolate     (1) -GGRCVASCEVGAVAKRILCFGDSLTWGWIPVEEGVPTQRFP-KRVRWTG
  M. parafortuitum CO1      (1) -------------MAKRILCFGDSLTWGWIPVEEGVPTERFP-RDVRWTG
                   MSAT     (1) -------------MAKRILCFGDSLTWGWVPVEDGAPTERFA-PDVRWTG
```

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```
                     Sm-RSM05666   (1) --------------MKTVLCYGDSLTWGYDATG-----SGRHALEDRWPS
                       At-Q8UAC0   (1) --------------MKTVLAFGDSLTWGADPAT-----GLRHPVEHRWPD
                       At-Q8UFG4   (1) -------------MVKSVLCFGDSLTWGSNAET-----GGRHSHDDLWPS
                     M091_M4aE11   (1) --------------MKTILAYGDSLTYGANPIP----GGPRHAYEDRWPT
                    M1-RMLO00301   (1) MAGGTRLDECTGERMKTVLCYGDSLTWGYNAE------GGRHALEDRWPS
            P.dejongeii RVM04532   (1) --------------MKTILCFGDSNTWGYDPASMTAPFPRRHGPEVRWTG
   Q92XZ1 Sinorhizobium meliloti   (1) ---------MEETVARTVLCFGDSNTHGQVPG--RGPLDRYR-REQRWGG
                    S261_M2aA12   (1) --------------MKNILAFGDSLTWGFVAG-----QDARHPFETRWPN
  Sma1993 Sinorhizobium meliloti   (1) MTINSHSWRTLMVEKRSVLCFGDSLTWGWIPVKESSPTLRYP-YEQRWTG
                     ZP_00197751   (1) --------------MKTILCYGDSLTWGYDAVG-----PSRHAYEDRWPS
                     ZP_00216984   (1) ----------MTMTQKTVLCYGDSNTHGTRPMTHAGGLGRFA-REERWTG
                        BAB16192   (1) -----MICHKGGEEMRSVLCYGDSNTHGQIPG--GSPLDRYG-PNERWPG
                        BAB16197   (1) -----------MAESRSILCFGDSLTWGWIPVPESSPTLRYP-FEQRWTG
                       NP_522806   (1) --------------MQQILLYSDSLSWGIIPG-----TRRRLPFAARWAG
                       Consensus   (1)               MKTILCFGDSLTWGWIPV    P  RR   E RW G

                                       51                                              100
                             20A  (37) VLADLLGDRYEVIE---EGLSARTTTADDPADPRLN-GSQYLPSCLASHL
              9B Natural Isolate .(49) VLADELGAGYEVVE---EGLSARTTTADDPTDPRLN-GSDYLPACLASHL
             M. parafortuitum CO1 (37) VLADLLGDRYEVIE---EGLSARTTTAEDPADPRLN-GSQYLPSCLASHL
                            MSAT  (37) VLAQQLGADFEVIE---EGLSARTTNIDDPTDPRLN-GASYLPSCLATHL
                     Sm-RSM05666  (32) VLQKALGSDAHVIA---EGLNGRTTAYDDHLADCDRNGARVLPTVLHTHA
                       At-Q8UAC0  (32) VLEAELAGKAKVHP---EGLGGRTTCYDDHAGPACRNGARALEVALSCHM
                       At-Q8UFG4  (33) VLQKALGSDVHVIFT-HEGLGGRTTAYDDHTGDCDRNGARLLPTLLHSHA
                     M091_M4aE11  (33) ALEQGLGGKARVIA---EGLGGRTTVHDDWFANADRNGARVLPTLLESHS
                    M1-RMLO00301  (45) VLQASLGGGVQVIA---DGLNGRTTAFDDHLAGADRNGARLLPTALTTHA
            P.dejongeii RVM04532  (37) VLAKALGAGFRVIE---EGQNGRTTVHEDPLNICRK-GKDYLPACLESHK
   Q92XZ1 Sinorhizobium meliloti  (39) VLQGLLGPNWQVIE---EGLSGRTTVHDDPIEGSLKNGRIYLRPCLQSHA
                     S261_M2aA12  (32) ALAAGLGGKARVIE---EGQNGRTTVFDDAATFESRNGSVALPLLLISHQ
  Sma1993 Sinorhizobium meliloti  (50) AMAARLGDGYHIIE---EGLSARTTSLDDPNDARLN-GSTYLPMALASHL
                     ZP_00197751  (32) VLQGRLGSSARVIA---EGLCGRTTAFDDWVAGADRNGARILPTLLATHS
                     ZP_00216984  (40) VLAQTLGASWRVIE---EGLPARTTVHDDPIEGRHKNGLSYLRACVESHL
                        BAB16192  (43) VLRRELGSQWYVIE---EGLSGRTTVRDDPIEGTMKNGRTYLRPCLMSHA
                        BAB16197  (39) AMAAALGDGYSIIE---EGLSARTTSVEDPNDPRLN-GSAYLPMALASHL
                       NP_522806  (32) VMEHALQAQGHAVRIVEDCLNGRTTVLDDPARPGRN-GLQGLAQRIEAHA
                       Consensus  (51) VLA  LGA Y VIE   EGL GRTT  DDP D   RNGA YLP  L SH

                                       101                                             150
                             20A  (83) PLDLVILMLGINDTKANFGRTPFD--IATGMGVLATQVLTSAGG-VGTSY
              9B Natural Isolate  (95) PLDLVILMLGTNDTKANLNRTPVD--IASGMGVLATQVLTSAGG-VGTSY
             M. parafortuitum CO1 (83) PLDLVILMLGTNDTKANFGRTPFD--IATGMGVLATQVLTSAGG-VGTSY
                            MSAT  (83) PLDLVIIMLGTNDTKAYFRRTPLD--IALGMSVLVTQVLTSAGG-VGTTY
                     Sm-RSM05666  (79) PLDLIVFMLGSNDMKPIIHGTAFG--AVKGIERLVNLVRRHDWPT--ETE
                       At-Q8UAC0  (79) PLDLVIIMLGTNDIKPVHGGRAEA--AVSGMRRLAQIVETFIYK---PRE
                       At-Q8UFG4  (82) PLDMVIIMLGTNDMKPAIHGSAIVAFTMKGVERLVKLTRNHVWQV--SDW
                     M091_M4aE11  (80) PLDLIVIMLGTNDIKPHHGRTAGE--AGRGMARLVQIIRGHYAG---RMQ
                    M1-RMLO00301  (92) PIDLIVIMLGANDMKPWIHGNPVA--AKQGIQRLIDIVRGHDYP---FDW
```

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```
          P.dejongeii RVM04532  (83)  PLDLVILMLGTNDLKSTFNVPPGE--IAAGAGVLGRMILAGDA---GPEN
 Q92XZ1 Sinorhizobium meliloti  (86)  PLDLIIIMLGTNDLKRRFNMPPSE--VAMGIGCLVHDIRELSP---GRTG
                  S261_M2aA12   (79)  PLDLVIIMLGTNDIKFAARCRAFD--ASMGMERLIQIVRSANYM---KGY
Sma1993 Sinorhizobium meliloti  (96)  PLDLVIIMLGTNDTKSYFHRTPYE--IANGMGKLVGQVLTCAGG-VGTPY
                  ZP_00197751   (79)  PLDLVIVMLGTNDMKSFVCGRAIG--AKQGMERIVQIIRGQPYS---FNY
                  ZP_00216984   (87)  PVDVVVLMLGTNDLKTRFSVTPAD--IATSVGVLLAKIAACGA---GPSG
                     BAB16192   (90)  ILDLVIIMLGTNDLKARFGQPPSE--VAMGIGCLVYDIRELAP---GPGG
                     BAB16197   (85)  PLDLVIILLGTNDTKSYFRRTPYE--IANGMGKLAGQVLTSAGG-IGTPY
                    NP_522806   (81)  PLALVIILMLGTNDFQAIFRHTAQD--AAQGVAQLVRAIRQAPIEP---GM
                    Consensus  (101)  PLDLVIIMLGTNDLKA F  TP D  IA GMGRLV  VR   G   G  Y

                                      151                                             200
                          20A  (130)  PAPQVLIVAPPPLGELPHPWFDL--VFSGGREKTAELARVYSALASFMKV
           9B Natural Isolate  (142)  PAPQVLIVAPPPLAEMPHPWFEL--VFDGGREKTAQLARVYSALASFMKV
         M. parafortuitum CO1  (130)  PAPQVLIVAPPPLGELPHPWFDL--VFSGGREKTAELARVYSALASFMKV
                         MSAT  (130)  PAPKVLVVSPPPLAPMPHPWFQL--IFEGGEQKTTELARVYSALASFMKV
                  Sm-RSM05666  (125)  EGPEILIVSPPPLCETANSAFAAMFAGGVEQSAMLAP--LYRDLADELDC
                   At-Q8UAC0   (124)  AVPKLLIVAPPPCVAGP--GGEPAGGRDIEQSMRLAP--LYRKLAAELGH
                   At-Q8UFG4   (130)  EAPDVLIVAPPQLCETANPFMGAIFRDAIDESAMLASVFTYRDLADELDC
                  M091_M4aE11  (125)  DEPQIILVSPPPIILGDWADMMDHFGPHEAIATSVDFAREYKKRADEQKV
                  M1-RMLO00301 (137)  PAPQILIVSPPVVSRTENADFREMFAGGDEASKQLAP--QYAALADEVGC
          P.dejongeii RVM04532 (128)  RPPQLLLMCPPKVRDLSAMPDLDAKIPHGAAR-SAEFPRHYKAQAVALKC
 Q92XZ1 Sinorhizobium meliloti (131)  NDPEIMIVAPPPMLEDLKEWES---IFSGAQEKSRKLALEFEIMADSLEA
                  S261_M2aA12  (124)  KIPEILIISPPSLVPTQDEWFNDLWGHAIAESKLFAK--HYKRVAEELKV
Sma1993 Sinorhizobium meliloti  (143)  PAPKVLVVAPPPLAPMPDPWFEG--MFGGGYEKSKELSGLYKALADFMKV
                  ZP_00197751  (124)  KVPSILLVAPPPLCATENSDFAEIFEGGMAESQKLAP--LYAALAQQTGC
                  ZP_00216984  (132)  ASPKLVLMAPAPIVEVGFLGEI---FAGGAAK-SRQLAKRYEQVASDAGA
                     BAB16192  (135)  KPPEIMVVAPPPMLDDIKEWEP---IFSGAQEKSRRLALEFEIIADSLEV
                     BAB16197  (132)  PAPKLLIVSPPPLAPMPDPWFEG--MFGGGYEKSLELAKQYKALANFLKV
                    NP_522806  (126)  PVPPVLIVVPPAITAPAGAMADK---FADAQPKCAGLAQAYRATAQTLGC
                    Consensus  (151)   AP ILIVAPPPL E    WF    IFGGA  KS  LA  YKALA  LKV

                                      201                                      248
                          20A  (178)  PFFDAGSVISTDGVDGTHFTRGETI------------------------ (SEQ ID NO:675)
           9B Natural Isolate  (190)  PFFDAGSVISTDGVDGTHFTRGETIDR---------------------- (SEQ ID NO:676)
         M. parafortuitum CO1  (178)  PFFDAGSVISTDGVDGIHFTRGEQST----------------------- (SEQ ID NO:677)
                         MSAT  (178)  PFFDAGSVISTDGVDGIHFTEANNRDLGVALAEQVRSLL---------- (SEQ ID NO:678)
                  Sm-RSM05666  (173)  GFFDGGSVARTTPIDGVHLDAENTRAVGRGLEPVVRMMLGL-------- (SEQ ID NO:679)
                    At-Q8UAC0  (170)  HFFDAGSVASASPVDGVHLDASATAAIGRALAAPVRDILG--------- (SEQ ID NO:680)
                    At-Q8UFG4  (180)  GFFDAGSVARTTPVDGVHLDAENTRAIGRGLEPVVRMMLGL-------- (SEQ ID NO:681)
                  M091_M4aE11  (175)  HFFDAGTVATTSKADGIHLDPANTRAIGAGLVPLVKQVLGL-------- (SEQ ID NO:682)
                  M1-RMLO00301 (185)  GFFDAGTVAQTTPLDGVHLDAENTRNIGKALTSVVRVML---------- (SEQ ID NO:683)
          P.dejongeii RVM04532 (177)  EYFNSQEIVETSPVDGIHLEASEHLKLGEALAEKVKVLLG--------- (SEQ ID NO:684)
 Q92XZ1 Sinorhizobium meliloti (178)  HFFDAGTVCQCSPADGFHIDEDAHRLLGEALAQEVLAIGWPDA------ (SEQ ID NO:685)
                  S261_M2aA12  (172)  HFFDAGTVAVADKTDGGHLDAVNTKAIGVALVPVVKSILAL-------- (SEQ ID NO:686)
Sma1993 Sinorhizobium meliloti  (191)  EFFAAGDCISTDGIDGIHLSAETNIRLGHAIADKVAALF---------- (SEQ ID NO:687
                  ZP_00197751  (172)  AFFDAGTVARTTPLDGIHLDAENTRAIGAGLEPVVRQALGL-------- (SEQ ID NO:688)
```

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```
ZP_00216984  (178) HFLDAGAIVEVSPVDGVHFAADQHRVLGQRVAALLQQIA--------- (SEQ ID NO:689)
   BAB16192  (182) HFFDAATVASCDPCDGFHINREAHEALGTALAREVEAIGWR------- (SEQ ID NO:690)
   BAB16197  (180) DFLDAGEFVKTDGCDGIHFSAETNITLGHAIAAKVEAIFSQEAKNAAA (SEQ ID NO:691)
  NP_522806  (173) HVFDANSVTPASRVDGIHLDADQHAQLGRAMAQVVGTLLAQ------- (SEQ ID NO:692)
  Consensus  (201)  FFDAGSV  TSPVDGIHLDAENTR LG ALA  VR IL          (SEQ ID NO:693)
```

The guide tree to the CLUSTALW alignment (which approximates to a phylogenetic tree) clearly indicates 3 groupings:

1) GDSL – ARTT group including Act

2) GDSL – GRTT group composed of members of the *Rhizobiales* and the metagenome; and

3) Intermediate group of mixed motifs.

It is also contemplated that the results suggest some form of gene duplication and mutation events in the *Rhizobiales* and lateral gene transfer to *Mycobacterium*.

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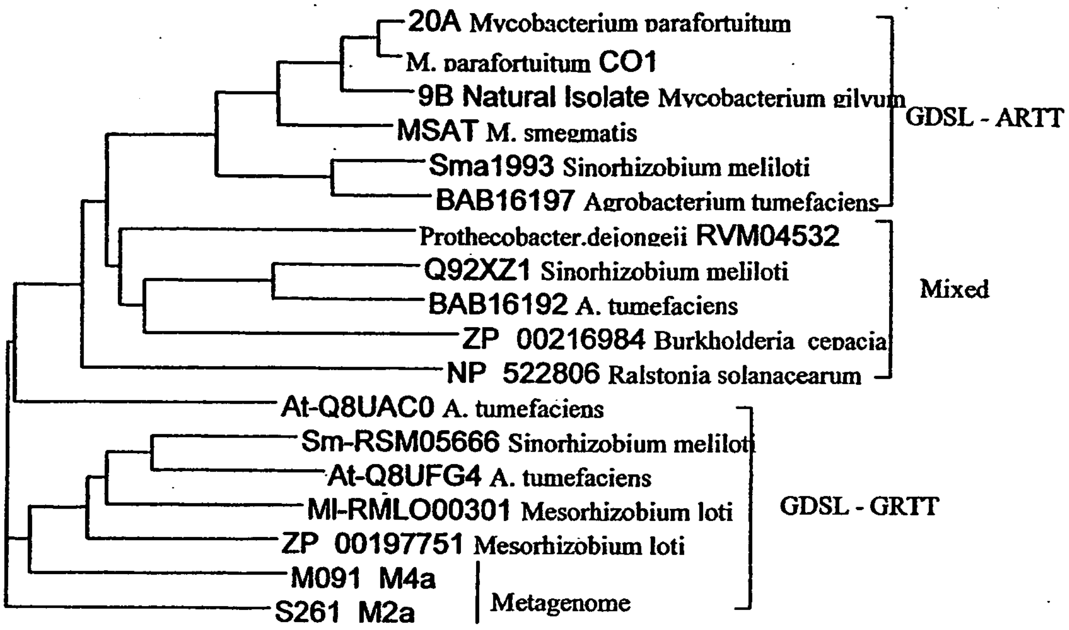

Using the non-redundant alignment a new Act consensus was constructed called "Act chimera".

```
  1   KTILCFGDSL TWGWIPVEDG APTERRAPEV RWTGVLAQQL GADYEVIEEG
 51   LSGRTTNIDD PTDPRLRNGA SYLPSCLASH LPLDLVIIML GTNDLKAYFR
101   RTPLDIALGM GRLVTQVRTS AGGVGTTYPA PKILIVAPPP LAEMPHPWFQ
151   LIFGGAEQKS TELARVYKAL ASFLKVPFFD AGSVISTSPV DGIHLDAENT
201   RDLGVALAEQ VRSIL  (SEQ ID NO:694)
```

An alignment of Act-chimera with Ms Act (Chimera align) indicates 91.6% similarity and 86.0% identity, as indicated below.

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```
                  1                                                50
       MSAT   (1) MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLAQQLGADFEVIE
Act-Chimera   (1) --KTILCFGDSLTWGWIPVEDGAPTERRAPEVRWTGVLAQQLGADYEVIE
  Consensus   (1)   K ILCFGDSLTWGWIPVEDGAPTER APDVRWTGVLAQQLGADFEVIE

                  51                                              100
       MSAT  (51) EGLSARTTNIDDPTDPRLN-GASYLPSCLATHLPLDLVIIMLGTNDTKAY
Act-Chimera  (49) EGLSGRTTNIDDPTDPRLRNGASYLPSCLASHLPLDLVIIMLGTNDLKAY
  Consensus  (51) EGLSARTTNIDDPTDPRL  GASYLPSCLASHLPLDLVIIMLGTND KAY

                  101                                             150
       MSAT (100) FRRTPLDIALGMSVLVTQVLTSAGGVGTTYPAPKVLVVSPPPLAPMPHPW
Act-Chimera  (99) FRRTPLDIALGMGRLVTQVRTSAGGVGTTYPAPKILIVAPPPLAEMPHPW
  Consensus (101) FRRTPLDIALGM  LVTQV TSAGGVGTTYPAPKILIVAPPPLA MPHPW

                  151                                             200
       MSAT (150) FQLIFEGGEQKTTELARVYSALASFMKVPFFDAGSVISTDGVDGIHFTEA
Act-Chimera (149) FQLIFGGAEQKSTELARVYKALASFLKVPFFDAGSVISTSPVDGIHLDAE
  Consensus (151) FQLIF GAEQKSTELARVY ALASFLKVPFFDAGSVIST  VDGIH

                  201             217
       MSAT (200) NNRDLGVALAEQVRSLL  (SEQ ID NO:695)
Act-Chimera (199) NTRDLGVALAEQVRSIL  (SEQ ID NO:694)
  Consensus (201) N RDLGVALAEQVRSIL  (SEQ ID NO:696)
```

A BLASTP search with Act-chimera did not reveal any further sequences.

The Act-chimera is "forced" on the Per sequence at the positions where no consensus exists. However, a basic 'unforced' consensus sequence did not provide any more information from a blastp search or from alignment analysis. Thus, comparison with the most distant homologues in the blastp 'hit' list was considered more useful in defining the important residues/positions in Act sequence space. This was a useful exercise, as these sequences were not used in the non-redundant alignment.

For example, *Rhodopirellula baltica* (NP_865748; Psp; a *Planctomycetes* and quite different from either *Mycobacterium* or *Rhizobiales*), was compared as shown below.

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```
                 1                                               50
      MSAT   (1) MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLA---QQLGADFE
 NP_865746   (1) -MHSILIYGDSLSWGIIPGTR----RRFAFHQRWPGVMEIELRQTGIDAR
 Consensus   (1)     IL FGDSLSWG IP        RFA   RW GVL     Q G D

                 51                                             100
      MSAT  (48) VIEEGLSARTTNIDDPTDPRLNGASYLPSCLATHLPLDLVIIMLGTNDTK
 NP_865746  (46) VIEDCLNGRRTVLEDPIKPGRNGLDGLQQRIEINSPLSLVVLFLGTNDFQ
 Consensus  (51) VIED L AR T IDDP  P  NG   L   I    PL LVII LGTND

                 101                                            150
      MSAT  (98) AYFRRTPLDIALGMSVLVTQVLTSAGGVGTTYPAPKVLVVSPPPLAPMPH
 NP_865746  (96) SVHEFHAEQSAQGLALLV--DAIRRSPFEPGMPTPKILLVAPPTVHH-PK
 Consensus (101) A         A GLALLV              P PKILLVAPP L   P

                 151                                            200
      MSAT (148) PWFQLIFEGGEQKTTELARVYSALASFMKVPFFDAGSVISTDGVDGIHFT
 NP_865746 (143) LDMAAKFQNAETKSTGLADAIRKVSTEHSCEFFDAATVTTTSVVDGVHLD
 Consensus (151)       F  AE KST LA     LAS     FFDAASV ST  VDGIH

                 201                222
      MSAT (198) EANNRDLGVALAEQVRSLL---  (SEQ ID NO:695)
 NP_865746 (193) QEQHQALGTALASTIAEILADC  (SEQ ID NO:697)
 Consensus (201)   N   LG ALA  I  IL     (SEQ ID NO:698)
```

The following is an alignment with *Ralstonia eutropha* (Reu):

```
                  1                                               50
       MSAT   (1) ---------MAKRILCFGDSLTWGWVPVEDGAPTERFAPDVRWTGVLA--
ZP_00166901   (1) MPLTAPSEVDPLQILVYADSLSWGIVPGTR----RRLPFPVRWPGRLELG
  Consensus   (1)              IL FADSLSWG VP        R    VRW G L

                  51                                             100
       MSAT  (40) --QQLGADFEVIEEGLSARTTNIDDPTDPRLNGASYLPSCLATHLPLDLV
ZP_00166901  (47) LNADGGAPVRIIEDCLNGRRTVWDDPFKPGRNGLQGLAQRIEIHSPVALV
  Consensus  (51)      GA   IIED L AR T  DDP  P  NG   L   I  H PL LV

                  101                                            150
       MSAT  (88) IIMLGTNDTKAYFRRTPLDIALGMSVLVTQVLTSAGGVGTTYPAPKVLVV
ZP_00166901  (97) VLMLGNNDFQSMHPHNAWHAAQGVGALV--HAIRTAPIEPGMPVPPILVV
  Consensus (101) IIMLG ND  A         A GM  LV       A I    P P ILVV
```

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```
                     151                                                200
        MSAT   (138) SPPPLAPMPHPWFQLIFEGGEQKTTELARVYSALASFMKVPFFDAGSVIS
 ZP_00166901   (145) VPPPIRT-PCGPLAPKFAGGEHKWAGLPEALRELCATVDCSLFDAGTVIQ
   Consensus   (151)  PPPI    P          F GGE K    L       L A M     FDAGSVI

                     201                                 237
        MSAT   (188) TDGVDGIHFTEANNRDLGVALAEQVRSLL-------- (SEQ ID NO:695)
 ZP_00166901   (194) SSAVDGVHLDADAHVALGDALQPVVRALLAESSGHPS (SEQ ID NO:699)
   Consensus   (201) S AVDGIH          LG AL    VRALL        (SEQ ID NO:700)
```

Based on these results, the following conclusions were made. A BLASTp nr-database search with a perhydrolase consensus sequence revealed GDSL or GDSI lipases/esterases from a wide diversity of organisms. However, only 12 or 14 of these were reliable homologues of Per. Nearly all of these were derived from 1 small group of bacteria, namely the *Rhizobiales* (*i.e.*, Gram-negative soil bacteria belonging the alpha-*Proteobacteria*). A few members of the beta-*Proteobacteria* were found, but no *Mycobacterium sp*. This provides an indication that the perhydrolase (Per) gene/protein is not widely distributed in nature.

The *Mycobacterium* protein is characterized by the GDSL-ARTT motif, whereas most of the *Rhizobiales* are characterized by a GDSL-GRTT motif. There are also some mixed or intermediate motifs (*e.g.*, GDSN-GRTT, GDSN-ARTT and SDSL-GRTT). This may indicate gene duplication and mutation event and lateral gene transfer. The consensus residues identified in these experiments were L6, W14, R27, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, and G205.

Using the non-redundant alignment and comparison with distant homologues the follow sequence space can be defined starting at position 5 of the *M. smegmatis* perhydrolase and ending at position 195, with perhydrolase shown in residues in bold.

[**I**, V][**L**][X][**F**, Y][**G**, S][**D**][**S**][**L**, N][**T**, S][**W**, Y, H][**G**][X]$_{2}$[**P**, A][X]$_{14}$[**R**, L][**W**]

[X]$_{7}$[**L**][X]$_{5}$[**V**, I][**I**, V, H][X][**E**. D][**G**, C][**L**, Q][X][**G**, A][**R**][**T**][**T**][X]$_{2}$[**D**, E][**D**]

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[X]$_{7}$[G][X]$_{3}$[L][X]$_{6}$[H][X][P, I][L, I, V][D, A][V, I][X]$_{2}$[M, L][L][G][X][N][D] [X]$_{36}$[P][X]$_{6}$[P][P, A][X]$_{31}$[A][X]$_{19}$[D][G][X][H] (SEQ ID NO:701)

In sum, it is clear from the analyses above that the active clones/sequences with a GDSx_1 – x_2RTT – Gx_3ND motif have all been found among the alpha-*Proteobacteria* – Gram-negative bacteria associated with the soil rhizosphere. This is in sharp contrast to the prototype perhydrolase from *M. smegmatis* – a high GC content Gram-positive bacterium assigned to the class *Actinobacteria*. This division is illustrated in Figure 2, which provides a phylogenetic tree, showing the major branches of the bacteria and the origin of the active clones/sequences compared to *M. smegmatis*.

EXAMPLE 14

Native Molecular Weight Estimation of Homologues of the Perhydrolase

In this Example, experiments conducted to estimate the native molecular weights of *M. smegmatis* perhydrolase homologues are described.

Preparation of Samples for Purification (Size Determination)

A single colony of the desired strains was inoculated in 50ml Terrific Broth and incubated overnight at 37°C with shaking at 200 rpm. The cells were pelleted by centrifugation for 10 minutes at 7000 rpm in a Sorvall SuperSpeed Centrifuge. The pellets were then resuspended in 10 ml 25mM Bis-Tris (pH 6.5) and lysed by passage through a French pressure cell twice. The lysates were then centrifuged at 15000 rpm in a Sorvall SuperSpeed Centrifuge. The soluble fraction was heat treated at 55°C for 1 hour to precipitate cellular proteins. The samples were then centrifuged at 10000 rpm in a Sorvall SuperSpeed Centrifuge and the soluble fractions used for further purification or assay.

Sizing Columns

The supernatants (prepared as described above) were run on a Sephadex 200 sizing column in 20 mM phosphate (pH 8.0), with a flow rate of 0.5 ml/min. The column was calibrated prior to running the samples with MW standards (listed below) and purified *M. smegmatis* perhydrolase protein. The crude sample elution volumes were determined by collecting 0.5 ml fractions, and assaying the fractions for pNB activity.

Molecular weights and elution volumes of the standards:

Thyroglobulin MW 669 kDa : elution volume 16ml

Aldolase MW 158 kDa: elution volume 24 ml

Ovalbumin MW 43 kDa: elution volume 26 ml

Ribonuclease MW 14 kDa: elution volume 32 ml

Perhydrolase elution volume 24 ml

Results

The following Table (Table 14-1) provides the elution volume of some of the *M. smegmatis* perhydrolase homologues identified herein.

Table 14-1. Elution Volume (Estimated Molecular Weight) of *M. smegmatis* Perhydrolase Homologues

Homologue Sample	Elution Volume (ml)
pLO_SmeI	24
pET26_SmeII	24
pET26_MlO	24
pET26b_Stm	24
pET26b_Mbo	24
M7OaEB_pET26	32
pET26_m2aA12	24
pET26b_S2487am	32

S. meliloti RSM02162 (G00355)	24
PET_M2aA12 (5261)	24
M. smegmatis Perhydrolase	24

The data in the above Table and the assay results obtained for these homologues indicated that these enzymes have an amino acid sequence similar to the *M. smegmatis* perhydrolase. As with the *M. smegmatis* perhydrolase, these homologues exhibit perhydrolysis activity as multimers. As described herein, the perhydrolase is an octamer, while the homologues, although they elute in a similar volume, are contemplated to be dimers, trimers, tetramers, hexamers, and/ or octamers.

EXAMPLE 15

Crystal Structure of Perhydrolase

In this Example, the crystallographic analysis of the perhydrolase is described. Perhydrolase crystals were obtained under two conditions: 2.0 M $[NH_4]_2SO_4$, 2% PEG400, 0.1 M Tris pH 7.1 (giving triclinic, P1 crystals) and 1.0 M ammonium dihydrogen phosphate, and 0.1M sodium citrate pH 5.6 (giving tetragonal, P4 crystals) Both crystal forms gave suitable diffraction beyond 2.0Å resolution. Derivative protein for a MAD phase determination using selenium replacing sulfur containing methionine resulting in a protein molecule having four selenomethionines the N-terminal methionine is cleaved proteolytically. Of the two forms, triclininc P1 a= 83.77Å b=90.07Å c= 112.115Å α =73.32° β = 77.30° γ = 88.07° and P4 a=b=98.18Å c= 230.12Å, the P4 crystal gave data that was possible to use for structure determination. Three wavelength MAD datasets were collected at wavelengths corresponding to the Se absorption edge, near the inflection point and a third, away from the absorption edge.

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Three hundred and thirty-three frames (0.3 degree oscillations per frame) for each wavelength with 1 sec exposure time were collected from a single tetragonal space group P4 crystal. The structure could be solved with either SOLVE or SHELX computer programs giving similar solutions for the 32 possible Se positions. The map was fitted using the program "O". It was possible to trace electron density for residues 3-216 in each of the eight independent molecules. The final structure of these eight molecules was refined using CNS. The current crystallographic R-factor is 21%. The coordinates are provided below.

```
CRYST1    98.184    98.184  230.119  90.00  90.00  90.00
SCALE1      0.010185  0.000000  0.000000        0.000000
SCALE2      0.000000  0.010185  0.000000        0.000000
SCALE3      0.000000  0.000000  0.004346        0.000000
ATOM      1  CB  LYS     3      -8.167 -61.964  18.588 1.000 40.95
ATOM      2  CG  LYS     3      -8.685 -63.192  19.323 1.000 22.95
ATOM      3  CD  LYS     3      -8.635 -64.400  18.399 1.000 14.97
ATOM      4  CE  LYS     3      -7.963 -65.575  19.090 1.000 19.83
ATOM      5  NZ  LYS     3      -7.359 -66.511  18.099 1.000 44.28
ATOM      6  C   LYS     3      -9.684 -60.377  17.426 1.000 13.89
ATOM      7  O   LYS     3      -9.087 -59.356  17.767 1.000 12.50
ATOM      8  N   LYS     3      -8.000 -61.626  16.153 1.000 15.57
ATOM      9  CA  LYS     3      -8.919 -61.686  17.284 1.000 20.71
ATOM     10  N   ARG     4     -10.987 -60.381  17.166 1.000 24.56
ATOM     11  CA  ARG     4     -11.695 -59.097  17.204 1.000 22.65
ATOM     12  CB  ARG     4     -12.299 -58.822  15.822 1.000 21.44
ATOM     13  CG  ARG     4     -11.232 -58.465  14.792 1.000 21.56
ATOM     14  CD  ARG     4     -11.845 -58.181  13.431 1.000 29.29
ATOM     15  NE  ARG     4     -11.660 -56.790  13.020 1.000 32.87
ATOM     16  CZ  ARG     4     -12.643 -56.013  12.585 1.000 30.24
ATOM     17  NH1 ARG     4     -13.879 -56.487  12.494 1.000 17.82
ATOM     18  NH2 ARG     4     -12.399 -54.760  12.229 1.000 44.53
ATOM     19  C   ARG     4     -12.735 -59.054  18.308 1.000 14.59
ATOM     20  O   ARG     4     -13.604 -59.909  18.456 1.000 18.72
ATOM     21  N   ILE     5     -12.639 -58.012  19.131 1.000 13.45
ATOM     22  CA  ILE     5     -13.549 -57.882  20.263 1.000 12.08
ATOM     23  CB  ILE     5     -12.747 -57.835  21.578 1.000 15.40
ATOM     24  CG2 ILE     5     -13.678 -57.677  22.765 1.000  5.80
ATOM     25  CG1 ILE     5     -11.811 -59.034  21.741 1.000 11.66
ATOM     26  CD1 ILE     5     -10.437 -58.632  22.232 1.000 19.35
ATOM     27  C   ILE     5     -14.420 -56.640  20.142 1.000  8.96
```

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ATOM	28	O	ILE	5	-13.905	-55.529	20.021	1.000	13.31
ATOM	29	N	LEU	6	-15.736	-56.833	20.169	1.000	13.04
ATOM	30	CA	LEU	6	-16.675	-55.728	20.059	1.000	8.54
ATOM	31	CB	LEU	6	-17.879	-56.087	19.178	1.000	7.42
ATOM	32	CG	LEU	6	-18.959	-54.996	19.120	1.000	14.12
ATOM	33	CD1	LEU	6	-18.446	-53.783	18.359	1.000	12.19
ATOM	34	CD2	LEU	6	-20.245	-55.512	18.494	1.000	27.94
ATOM	35	C	LEU	6	-17.170	-55.293	21.436	1.000	2.72
ATOM	36	O	LEU	6	-17.719	-56.101	22.179	1.000	13.36
ATOM	37	N	CYS	7	-16.978	-54.020	21.756	1.000	1.38
ATOM	38	CA	CYS	7	-17.472	-53.469	23.011	1.000	3.17
ATOM	39	CB	CYS	7	-16.411	-52.582	23.667	1.000	7.01
ATOM	40	SG	CYS	7	-14.867	-53.471	23.992	1.000	11.21
ATOM	41	C	CYS	7	-18.755	-52.685	22.776	1.000	0.65
ATOM	42	O	CYS	7	-18.756	-51.627	22.145	1.000	4.76
ATOM	43	N	PHE	8	-19.859	-53.228	23.281	1.000	0.00
ATOM	44	CA	PHE	8	-21.147	-52.568	23.053	1.000	1.14
ATOM	45	CB	PHE	8	-22.115	-53.578	22.443	1.000	5.54
ATOM	46	CG	PHE	8	-23.421	-53.000	21.937	1.000	3.36
ATOM	47	CD1	PHE	8	-23.456	-52.212	20.800	1.000	0.89
ATOM	48	CD2	PHE	8	-24.602	-53.262	22.614	1.000	1.39
ATOM	49	CE1	PHE	8	-24.644	-51.683	20.333	1.000	0.00
ATOM	50	CE2	PHE	8	-25.793	-52.733	22.148	1.000	4.42
ATOM	51	CZ	PHE	8	-25.818	-51.944	21.012	1.000	2.71
ATOM	52	C	PHE	8	-21.677	-51.978	24.346	1.000	4.46
ATOM	53	O	PHE	8	-21.873	-52.672	25.348	1.000	6.98
ATOM	54	N	GLY	9	-21.923	-50.666	24.384	1.000	5.61
ATOM	55	CA	GLY	9	-22.396	-50.109	25.646	1.000	5.44
ATOM	56	C	GLY	9	-22.860	-48.673	25.522	1.000	5.66
ATOM	57	O	GLY	9	-23.229	-48.222	24.440	1.000	14.54
ATOM	58	N	ASP	10	-22.837	-47.964	26.641	1.000	3.89
ATOM	59	CA	ASP	10	-23.322	-46.596	26.734	1.000	5.17
ATOM	60	CB	ASP	10	-24.331	-46.467	27.880	1.000	2.99
ATOM	61	CG	ASP	10	-23.807	-47.052	29.175	1.000	7.05
ATOM	62	OD1	ASP	10	-22.617	-46.829	29.494	1.000	17.93
ATOM	63	OD2	ASP	10	-24.564	-47.738	29.895	1.000	10.98
ATOM	64	C	ASP	10	-22.154	-45.642	26.939	1.000	5.15
ATOM	65	O	ASP	10	-21.022	-45.940	26.556	1.000	5.62
ATOM	66	N	SER	11	-22.423	-44.497	27.554	1.000	9.02
ATOM	67	CA	SER	11	-21.394	-43.493	27.802	1.000	3.43
ATOM	68	CB	SER	11	-22.014	-42.331	28.585	1.000	7.25
ATOM	69	OG	SER	11	-22.640	-42.813	29.763	1.000	18.93
ATOM	70	C	SER	11	-20.199	-44.046	28.561	1.000	7.58
ATOM	71	O	SER	11	-19.089	-43.508	28.501	1.000	16.71
ATOM	72	N	LEU	12	-20.393	-45.133	29.308	1.000	6.56
ATOM	73	CA	LEU	12	-19.264	-45.696	30.046	1.000	16.41
ATOM	74	CB	LEU	12	-19.711	-46.759	31.042	1.000	17.05

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ATOM	75	CG	LEU	12	-20.598	-46.336	32.210	1.000	18.22
ATOM	76	CD1	LEU	12	-20.866	-47.527	33.123	1.000	7.48
ATOM	77	CD2	LEU	12	-19.973	-45.184	32.988	1.000	10.83
ATOM	78	C	LEU	12	-18.269	-46.285	29.048	1.000	14.99
ATOM	79	O	LEU	12	-17.065	-46.307	29.267	1.000	6.10
ATOM	80	N	THR	13	-18.828	-46.764	27.940	1.000	14.77
ATOM	81	CA	THR	13	-18.014	-47.347	26.876	1.000	8.83
ATOM	82	CB	THR	13	-18.828	-48.381	26.080	1.000	6.87
ATOM	83	OG1	THR	13	-19.109	-49.487	26.949	1.000	10.08
ATOM	84	CG2	THR	13	-18.033	-48.940	24.914	1.000	16.85
ATOM	85	C	THR	13	-17.490	-46.245	25.970	1.000	4.56
ATOM	86	O	THR	13	-16.315	-46.220	25.616	1.000	11.71
ATOM	87	N	TRP	14	-18.376	-45.317	25.612	1.000	5.57
ATOM	88	CA	TRP	14	-17.992	-44.210	24.742	1.000	7.21
ATOM	89	CB	TRP	14	-19.208	-43.329	24.453	1.000	6.90
ATOM	90	CG	TRP	14	-18.917	-42.183	23.537	1.000	11.88
ATOM	91	CD2	TRP	14	-18.731	-40.813	23.924	1.000	13.72
ATOM	92	CE2	TRP	14	-18.483	-40.081	22.745	1.000	11.95
ATOM	93	CE3	TRP	14	-18.752	-40.147	25.152	1.000	10.63
ATOM	94	CD1	TRP	14	-18.779	-42.222	22.181	1.000	8.28
ATOM	95	NE1	TRP	14	-18.517	-40.963	21.694	1.000	7.16
ATOM	96	CZ2	TRP	14	-18.255	-38.705	22.763	1.000	5.39
ATOM	97	CZ3	TRP	14	-18.526	-38.783	25.168	1.000	12.55
ATOM	98	CH2	TRP	14	-18.282	-38.084	23.981	1.000	12.81
ATOM	99	C	TRP	14	-16.880	-43.353	25.327	1.000	5.41
ATOM	100	O	TRP	14	-16.107	-42.745	24.582	1.000	4.90
ATOM	101	N	GLY	15	-16.794	-43.283	26.652	1.000	8.94
ATOM	102	CA	GLY	15	-15.794	-42.475	27.318	1.000	4.51
ATOM	103	C	GLY	15	-16.249	-41.098	27.755	1.000	10.98
ATOM	104	O	GLY	15	-15.480	-40.136	27.646	1.000	15.11
ATOM	105	N	TRP	16	-17.471	-40.952	28.255	1.000	23.34
ATOM	106	CA	TRP	16	-17.988	-39.691	28.792	1.000	15.10
ATOM	107	CB	TRP	16	-19.408	-39.890	29.327	1.000	6.11
ATOM	108	CG	TRP	16	-20.139	-38.694	29.846	1.000	1.78
ATOM	109	CD2	TRP	16	-21.229	-38.008	29.213	1.000	8.98
ATOM	110	CE2	TRP	16	-21.613	-36.942	30.051	1.000	7.76
ATOM	111	CE3	TRP	16	-21.923	-38.186	28.009	1.000	15.66
ATOM	112	CD1	TRP	16	-19.927	-38.021	31.016	1.000	0.35
ATOM	113	NE1	TRP	16	-20.798	-36.973	31.154	1.000	8.35
ATOM	114	CZ2	TRP	16	-22.649	-36.063	29.734	1.000	5.16
ATOM	115	CZ3	TRP	16	-22.952	-37.317	27.692	1.000	5.34
ATOM	116	CH2	TRP	16	-23.306	-36.269	28.551	1.000	4.72
ATOM	117	C	TRP	16	-17.059	-39.154	29.881	1.000	7.85
ATOM	118	O	TRP	16	-16.846	-39.815	30.899	1.000	3.97
ATOM	119	N	VAL	17	-16.533	-37.952	29.685	1.000	5.45
ATOM	120	CA	VAL	17	-15.750	-37.256	30.695	1.000	12.08
ATOM	121	CB	VAL	17	-14.822	-36.191	30.082	1.000	17.55

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ATOM	122	CG1	VAL	17	-14.084	-35.443	31.185	1.000	11.59
ATOM	123	CG2	VAL	17	-13.841	-36.807	29.099	1.000	7.77
ATOM	124	C	VAL	17	-16.673	-36.565	31.696	1.000	13.86
ATOM	125	O	VAL	17	-17.390	-35.618	31.351	1.000	1.02
ATOM	126	N	PRO	18	-16.660	-37.034	32.936	1.000	8.38
ATOM	127	CD	PRO	18	-15.770	-38.071	33.476	1.000	8.64
ATOM	128	CA	PRO	18	-17.572	-36.501	33.948	1.000	9.99
ATOM	129	CB	PRO	18	-17.201	-37.294	35.208	1.000	12.31
ATOM	130	CG	PRO	18	-15.817	-37.789	34.954	1.000	7.46
ATOM	131	C	PRO	18	-17.327	-35.017	34.191	1.000	13.05
ATOM	132	O	PRO	18	-16.163	-34.619	34.306	1.000	18.63
ATOM	133	N	VAL	19	-18.381	-34.211	34.266	1.000	6.92
ATOM	134	CA	VAL	19	-18.214	-32.793	34.585	1.000	9.29
ATOM	135	CB	VAL	19	-18.482	-31.856	33.388	1.000	5.33
ATOM	136	CG1	VAL	19	-17.377	-31.995	32.354	1.000	6.78
ATOM	137	CG2	VAL	19	-19.850	-32.150	32.796	1.000	3.72
ATOM	138	C	VAL	19	-19.151	-32.380	35.710	1.000	12.02
ATOM	139	O	VAL	19	-20.217	-32.962	35.913	1.000	14.52
ATOM	140	N	GLU	20	-18.771	-31.351	36.467	1.000	17.17
ATOM	141	CA	GLU	20	-19.662	-30.994	37.575	1.000	13.30
ATOM	142	CB	GLU	20	-18.918	-30.130	38.595	1.000	25.34
ATOM	143	CG	GLU	20	-18.276	-30.968	39.702	1.000	31.46
ATOM	144	CD	GLU	20	-16.871	-30.487	40.017	1.000	35.91
ATOM	145	OE1	GLU	20	-16.143	-30.157	39.055	1.000	40.11
ATOM	146	OE2	GLU	20	-16.507	-30.431	41.210	1.000	45.47
ATOM	147	C	GLU	20	-20.913	-30.294	37.080	1.000	7.56
ATOM	148	O	GLU	20	-21.964	-30.361	37.723	1.000	11.30
ATOM	149	N	ASP	21	-20.852	-29.610	35.936	1.000	19.38
ATOM	150	CA	ASP	21	-22.099	-28.983	35.471	1.000	23.47
ATOM	151	CB	ASP	21	-21.815	-27.740	34.640	1.000	17.53
ATOM	152	CG	ASP	21	-21.114	-27.991	33.326	1.000	14.93
ATOM	153	OD1	ASP	21	-20.984	-29.159	32.908	1.000	26.78
ATOM	154	OD2	ASP	21	-20.685	-26.996	32.694	1.000	8.74
ATOM	155	C	ASP	21	-22.959	-29.988	34.707	1.000	19.54
ATOM	156	O	ASP	21	-23.988	-29.627	34.131	1.000	22.49
ATOM	157	N	GLY	22	-22.550	-31.250	34.697	1.000	13.19
ATOM	158	CA	GLY	22	-23.279	-32.377	34.166	1.000	15.71
ATOM	159	C	GLY	22	-23.507	-32.377	32.659	1.000	20.02
ATOM	160	O	GLY	22	-23.370	-33.431	32.036	1.000	23.32
ATOM	161	N	ALA	23	-23.846	-31.235	32.138	1.000	26.40
ATOM	162	CA	ALA	23	-24.265	-30.672	30.873	1.000	28.79
ATOM	163	CB	ALA	23	-24.483	-29.192	31.152	1.000	32.86
ATOM	164	C	ALA	23	-23.309	-30.988	29.745	1.000	22.68
ATOM	165	O	ALA	23	-22.922	-32.189	29.753	1.000	40.02
ATOM	166	N	PRO	24	-22.847	-30.255	28.748	1.000	12.97
ATOM	167	CD	PRO	24	-22.892	-28.855	28.309	1.000	15.92
ATOM	168	CA	PRO	24	-22.051	-31.028	27.767	1.000	5.31

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ATOM	169	CB	PRO	24	-22.024	-30.134	26.520	1.000	4.03
ATOM	170	CG	PRO	24	-22.002	-28.762	27.105	1.000	6.80
ATOM	171	C	PRO	24	-20.622	-31.273	28.222	1.000	14.45
ATOM	172	O	PRO	24	-20.034	-30.591	29.056	1.000	19.65
ATOM	173	N	THR	25	-20.062	-32.310	27.600	1.000	13.21
ATOM	174	CA	THR	25	-18.685	-32.690	27.894	1.000	11.82
ATOM	175	CB	THR	25	-18.691	-33.772	28.987	1.000	12.19
ATOM	176	OG1	THR	25	-17.348	-34.104	29.355	1.000	19.38
ATOM	177	CG2	THR	25	-19.372	-35.027	28.454	1.000	0.00
ATOM	178	C	THR	25	-18.009	-33.160	26.620	1.000	14.10
ATOM	179	O	THR	25	-18.555	-33.019	25.518	1.000	16.46
ATOM	180	N	GLU	26	-16.818	-33.724	26.762	1.000	12.30
ATOM	181	CA	GLU	26	-16.157	-34.314	25.598	1.000	13.24
ATOM	182	CB	GLU	26	-14.909	-33.518	25.225	1.000	15.75
ATOM	183	CG	GLU	26	-15.211	-32.066	24.873	1.000	25.45
ATOM	184	CD	GLU	26	-15.451	-31.152	26.056	1.000	27.41
ATOM	185	OE1	GLU	26	-14.687	-31.210	27.048	1.000	22.86
ATOM	186	OE2	GLU	26	-16.416	-30.347	26.012	1.000	17.32
ATOM	187	C	GLU	26	-15.850	-35.775	25.891	1.000	8.80
ATOM	188	O	GLU	26	-16.279	-36.316	26.909	1.000	2.55
ATOM	189	N	ARG	27	-15.121	-36.421	25.001	1.000	13.28
ATOM	190	CA	ARG	27	-14.783	-37.838	25.124	1.000	12.71
ATOM	191	CB	ARG	27	-14.857	-38.447	23.726	1.000	6.07
ATOM	192	CG	ARG	27	-14.491	-39.908	23.585	1.000	4.38
ATOM	193	CD	ARG	27	-14.879	-40.387	22.186	1.000	11.29
ATOM	194	NE	ARG	27	-14.974	-41.840	22.110	1.000	13.10
ATOM	195	CZ	ARG	27	-15.191	-42.517	20.992	1.000	9.74
ATOM	196	NH1	ARG	27	-15.337	-41.868	19.842	1.000	11.38
ATOM	197	NH2	ARG	27	-15.262	-43.839	21.029	1.000	0.00
ATOM	198	C	ARG	27	-13.413	-38.031	25.746	1.000	8.79
ATOM	199	O	ARG	27	-12.534	-37.181	25.579	1.000	17.59
ATOM	200	N	PHE	28	-13.183	-39.133	26.461	1.000	12.29
ATOM	201	CA	PHE	28	-11.826	-39.379	26.955	1.000	9.91
ATOM	202	CB	PHE	28	-11.783	-40.575	27.900	1.000	10.13
ATOM	203	CG	PHE	28	-12.084	-40.263	29.355	1.000	11.54
ATOM	204	CD1	PHE	28	-11.250	-39.431	30.084	1.000	8.88
ATOM	205	CD2	PHE	28	-13.194	-40.802	29.979	1.000	11.27
ATOM	206	CE1	PHE	28	-11.535	-39.156	31.408	1.000	8.90
ATOM	207	CE2	PHE	28	-13.486	-40.533	31.305	1.000	5.41
ATOM	208	CZ	PHE	28	-12.647	-39.703	32.020	1.000	0.61
ATOM	209	C	PHE	28	-10.901	-39.635	25.770	1.000	11.56
ATOM	210	O	PHE	28	-11.370	-40.112	24.736	1.000	13.14
ATOM	211	N	ALA	29	-9.612	-39.349	25.896	1.000	13.02
ATOM	212	CA	ALA	29	-8.674	-39.656	24.818	1.000	13.91
ATOM	213	CB	ALA	29	-7.275	-39.163	25.151	1.000	6.49
ATOM	214	C	ALA	29	-8.662	-41.157	24.545	1.000	15.68
ATOM	215	O	ALA	29	-8.937	-41.954	25.446	1.000	31.74

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ATOM	216	N	PRO	30	-8.345	-41.537	23.314	1.000	11.44
ATOM	217	CD	PRO	30	-7.982	-40.660	22.192	1.000	12.10
ATOM	218	CA	PRO	30	-8.326	-42.955	22.936	1.000	18.85
ATOM	219	CB	PRO	30	-7.822	-42.956	21.494	1.000	16.38
ATOM	220	CG	PRO	30	-7.283	-41.593	21.244	1.000	14.74
ATOM	221	C	PRO	30	-7.386	-43.767	23.826	1.000	13.40
ATOM	222	O	PRO	30	-7.570	-44.969	23.979	1.000	8.18
ATOM	223	N	ASP	31	-6.396	-43.115	24.412	1.000	22.50
ATOM	224	CA	ASP	31	-5.426	-43.715	25.312	1.000	26.63
ATOM	225	CB	ASP	31	-4.170	-42.841	25.398	1.000	30.41
ATOM	226	CG	ASP	31	-3.792	-42.143	24.108	1.000	39.21
ATOM	227	OD1	ASP	31	-2.577	-42.086	23.802	1.000	39.00
ATOM	228	OD2	ASP	31	-4.673	-41.634	23.375	1.000	37.50
ATOM	229	C	ASP	31	-5.985	-43.926	26.721	1.000	17.49
ATOM	230	O	ASP	31	-5.482	-44.784	27.450	1.000	25.27
ATOM	231	N	VAL	32	-6.989	-43.150	27.092	1.000	14.45
ATOM	232	CA	VAL	32	-7.592	-43.125	28.421	1.000	12.64
ATOM	233	CB	VAL	32	-7.966	-41.683	28.814	1.000	10.68
ATOM	234	CG1	VAL	32	-8.580	-41.609	30.199	1.000	13.66
ATOM	235	CG2	VAL	32	-6.742	-40.774	28.752	1.000	20.51
ATOM	236	C	VAL	32	-8.808	-44.042	28.507	1.000	9.73
ATOM	237	O	VAL	32	-8.890	-44.834	29.452	1.000	2.23
ATOM	238	N	ARG	33	-9.722	-43.964	27.553	1.000	10.63
ATOM	239	CA	ARG	33	-10.888	-44.824	27.410	1.000	6.85
ATOM	240	CB	ARG	33	-11.369	-44.833	25.961	1.000	16.41
ATOM	241	CG	ARG	33	-12.281	-43.727	25.488	1.000	21.19
ATOM	242	CD	ARG	33	-12.464	-43.806	23.974	1.000	26.66
ATOM	243	NE	ARG	33	-11.862	-42.659	23.309	1.000	30.35
ATOM	244	CZ	ARG	33	-11.493	-42.567	22.044	1.000	31.60
ATOM	245	NH1	ARG	33	-11.658	-43.585	21.214	1.000	34.85
ATOM	246	NH2	ARG	33	-10.952	-41.433	21.610	1.000	52.70
ATOM	247	C	ARG	33	-10.600	-46.279	27.775	1.000	9.71
ATOM	248	O	ARG	33	-9.603	-46.830	27.300	1.000	16.85
ATOM	249	N	TRP	34	-11.450	-46.924	28.577	1.000	10.64
ATOM	250	CA	TRP	34	-11.166	-48.311	28.952	1.000	6.46
ATOM	251	CB	TRP	34	-12.149	-48.855	29.979	1.000	12.45
ATOM	252	CG	TRP	34	-13.561	-49.106	29.583	1.000	6.95
ATOM	253	CD2	TRP	34	-14.104	-50.199	28.835	1.000	9.27
ATOM	254	CE2	TRP	34	-15.493	-49.986	28.723	1.000	5.43
ATOM	255	CE3	TRP	34	-13.571	-51.345	28.240	1.000	14.72
ATOM	256	CD1	TRP	34	-14.622	-48.298	29.888	1.000	4.49
ATOM	257	NE1	TRP	34	-15.786	-48.820	29.374	1.000	4.03
ATOM	258	CZ2	TRP	34	-16.337	-50.864	28.050	1.000	8.19
ATOM	259	CZ3	TRP	34	-14.405	-52.216	27.572	1.000	12.73
ATOM	260	CH2	TRP	34	-15.778	-51.976	27.479	1.000	8.32
ATOM	261	C	TRP	34	-11.111	-49.214	27.723	1.000	7.27
ATOM	262	O	TRP	34	-10.393	-50.222	27.767	1.000	11.53

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ATOM    263  N   THR   35     -11.839 -48.887  26.659 1.000  1.15
ATOM    264  CA  THR   35     -11.730 -49.673  25.431 1.000  5.29
ATOM    265  CB  THR   35     -12.708 -49.239  24.331 1.000  3.10
ATOM    266  OG1 THR   35     -12.629 -47.820  24.163 1.000 15.85
ATOM    267  CG2 THR   35     -14.146 -49.549  24.726 1.000  5.16
ATOM    268  C   THR   35     -10.307 -49.555  24.882 1.000 14.32
ATOM    269  O   THR   35      -9.738 -50.494  24.333 1.000 12.77
ATOM    270  N   GLY   36      -9.756 -48.361  25.060 1.000 15.72
ATOM    271  CA  GLY   36      -8.392 -48.056  24.689 1.000 15.87
ATOM    272  C   GLY   36      -7.407 -48.785  25.583 1.000 14.86
ATOM    273  O   GLY   36      -6.374 -49.252  25.101 1.000 22.97
ATOM    274  N   VAL   37      -7.686 -48.905  26.884 1.000 12.48
ATOM    275  CA  VAL   37      -6.696 -49.577  27.728 1.000 11.76
ATOM    276  CB  VAL   37      -6.921 -49.365  29.229 1.000 10.95
ATOM    277  CG1 VAL   37      -6.092 -50.382  30.009 1.000  0.00
ATOM    278  CG2 VAL   37      -6.577 -47.940  29.630 1.000 10.31
ATOM    279  C   VAL   37      -6.707 -51.081  27.471 1.000 16.75
ATOM    280  O   VAL   37      -5.669 -51.735  27.494 1.000 14.29
ATOM    281  N   LEU   38      -7.911 -51.586  27.238 1.000 14.60
ATOM    282  CA  LEU   38      -8.094 -52.999  26.917 1.000 11.25
ATOM    283  CB  LEU   38      -9.573 -53.266  26.660 1.000 12.92
ATOM    284  CG  LEU   38      -9.975 -54.663  26.198 1.000 15.77
ATOM    285  CD1 LEU   38      -9.747 -55.691  27.293 1.000  0.00
ATOM    286  CD2 LEU   38     -11.425 -54.677  25.733 1.000 24.28
ATOM    287  C   LEU   38      -7.224 -53.347  25.720 1.000  7.67
ATOM    288  O   LEU   38      -6.408 -54.262  25.740 1.000 13.04
ATOM    289  N   ALA   39      -7.404 -52.568  24.659 1.000  9.64
ATOM    290  CA  ALA   39      -6.603 -52.667  23.451 1.000  3.53
ATOM    291  CB  ALA   39      -6.894 -51.487  22.530 1.000  6.32
ATOM    292  C   ALA   39      -5.112 -52.704  23.761 1.000  9.32
ATOM    293  O   ALA   39      -4.411 -53.632  23.367 1.000 28.59
ATOM    294  N   GLN   40      -4.653 -51.665  24.456 1.000 21.51
ATOM    295  CA  GLN   40      -3.251 -51.553  24.833 1.000 18.93
ATOM    296  CB  GLN   40      -2.974 -50.365  25.744 1.000 28.00
ATOM    297  CG  GLN   40      -3.597 -49.034  25.378 1.000 37.51
ATOM    298  CD  GLN   40      -3.070 -47.877  26.214 1.000 40.85
ATOM    299  OE1 GLN   40      -1.998 -47.335  25.933 1.000 61.34
ATOM    300  NE2 GLN   40      -3.809 -47.475  27.248 1.000  9.83
ATOM    301  C   GLN   40      -2.822 -52.851  25.525 1.000 10.96
ATOM    302  O   GLN   40      -1.856 -53.475  25.106 1.000 18.66
ATOM    303  N   GLN   41      -3.563 -53.239  26.552 1.000 15.02
ATOM    304  CA  GLN   41      -3.253 -54.423  27.337 1.000 22.27
ATOM    305  CB  GLN   41      -4.258 -54.582  28.484 1.000 16.69
ATOM    306  CG  GLN   41      -4.064 -53.605  29.624 1.000 14.55
ATOM    307  CD  GLN   41      -2.788 -53.852  30.406 1.000 16.86
ATOM    308  OE1 GLN   41      -2.759 -54.650  31.344 1.000 13.75
ATOM    309  NE2 GLN   41      -1.731 -53.158  30.008 1.000 21.79
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ATOM	310	C	GLN	41	-3.261	-55.694	26.493	1.000	28.40
ATOM	311	O	GLN	41	-2.442	-56.589	26.703	1.000	26.71
ATOM	312	N	LEU	42	-4.190	-55.776	25.546	1.000	28.62
ATOM	313	CA	LEU	42	-4.373	-57.007	24.780	1.000	26.50
ATOM	314	CB	LEU	42	-5.707	-56.920	24.012	1.000	19.31
ATOM	315	CG	LEU	42	-6.934	-57.122	24.914	1.000	16.32
ATOM	316	CD1	LEU	42	-8.226	-57.077	24.119	1.000	10.94
ATOM	317	CD2	LEU	42	-6.810	-58.438	25.673	1.000	15.03
ATOM	318	C	LEU	42	-3.217	-57.312	23.846	1.000	23.29
ATOM	319	O	LEU	42	-2.770	-58.457	23.728	1.000	20.82
ATOM	320	N	GLY	43	-2.693	-56.312	23.141	1.000	22.18
ATOM	321	CA	GLY	43	-1.605	-56.590	22.215	1.000	18.95
ATOM	322	C	GLY	43	-2.086	-56.793	20.791	1.000	23.97
ATOM	323	O	GLY	43	-3.284	-56.838	20.514	1.000	27.50
ATOM	324	N	ALA	44	-1.136	-56.927	19.879	1.000	22.72
ATOM	325	CA	ALA	44	-1.317	-57.012	18.448	1.000	24.25
ATOM	326	CB	ALA	44	0.048	-56.939	17.755	1.000	13.44
ATOM	327	C	ALA	44	-2.034	-58.272	17.990	1.000	23.83
ATOM	328	O	ALA	44	-2.146	-58.520	16.787	1.000	17.77
ATOM	329	N	ASP	45	-2.524	-59.086	18.917	1.000	21.59
ATOM	330	CA	ASP	45	-3.230	-60.298	18.495	1.000	17.80
ATOM	331	CB	ASP	45	-2.705	-61.491	19.296	1.000	18.22
ATOM	332	CG	ASP	45	-1.201	-61.625	19.113	1.000	24.69
ATOM	333	OD1	ASP	45	-0.710	-61.174	18.053	1.000	34.10
ATOM	334	OD2	ASP	45	-0.517	-62.159	20.007	1.000	33.14
ATOM	335	C	ASP	45	-4.732	-60.107	18.647	1.000	11.82
ATOM	336	O	ASP	45	-5.535	-60.992	18.364	1.000	23.89
ATOM	337	N	PHE	46	-5.097	-58.914	19.097	1.000	9.27
ATOM	338	CA	PHE	46	-6.485	-58.519	19.253	1.000	12.25
ATOM	339	CB	PHE	46	-6.909	-58.479	20.722	1.000	14.52
ATOM	340	CG	PHE	46	-6.474	-59.693	21.529	1.000	11.99
ATOM	341	CD1	PHE	46	-5.160	-59.814	21.956	1.000	12.17
ATOM	342	CD2	PHE	46	-7.383	-60.690	21.846	1.000	8.34
ATOM	343	CE1	PHE	46	-4.760	-60.917	22.683	1.000	13.46
ATOM	344	CE2	PHE	46	-6.990	-61.794	22.575	1.000	6.30
ATOM	345	CZ	PHE	46	-5.680	-61.904	22.998	1.000	8.44
ATOM	346	C	PHE	46	-6.725	-57.149	18.615	1.000	13.30
ATOM	347	O	PHE	46	-5.816	-56.366	18.366	1.000	27.22
ATOM	348	N	GLU	47	-7.992	-56.883	18.349	1.000	12.78
ATOM	349	CA	GLU	47	-8.469	-55.616	17.833	1.000	9.15
ATOM	350	CB	GLU	47	-8.667	-55.644	16.325	1.000	11.20
ATOM	351	CG	GLU	47	-8.791	-54.276	15.670	1.000	21.84
ATOM	352	CD	GLU	47	-9.726	-54.293	14.474	1.000	25.88
ATOM	353	OE1	GLU	47	-9.575	-55.205	13.632	1.000	30.74
ATOM	354	OE2	GLU	47	-10.602	-53.408	14.388	1.000	7.59
ATOM	355	C	GLU	47	-9.781	-55.280	18.550	1.000	11.37
ATOM	356	O	GLU	47	-10.722	-56.071	18.545	1.000	11.73

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ATOM	357	N	VAL	48	-9.775	-54.103	19.160	1.000	10.53
ATOM	358	CA	VAL	48	-10.954	-53.604	19.843	1.000	8.11
ATOM	359	CB	VAL	48	-10.595	-52.826	21.115	1.000	9.71
ATOM	360	CG1	VAL	48	-11.842	-52.251	21.773	1.000	15.31
ATOM	361	CG2	VAL	48	-9.849	-53.732	22.085	1.000	7.41
ATOM	362	C	VAL	48	-11.745	-52.714	18.882	1.000	12.72
ATOM	363	O	VAL	48	-11.147	-51.879	18.203	1.000	10.16
ATOM	364	N	ILE	49	-13.046	-52.943	18.862	1.000	13.04
ATOM	365	CA	ILE	49	-14.031	-52.170	18.122	1.000	14.10
ATOM	366	CB	ILE	49	-14.879	-53.068	17.203	1.000	16.77
ATOM	367	CG2	ILE	49	-15.735	-52.214	16.285	1.000	1.57
ATOM	368	CG1	ILE	49	-14.049	-54.081	16.415	1.000	18.10
ATOM	369	CD1	ILE	49	-14.687	-54.559	15.133	1.000	14.33
ATOM	370	C	ILE	49	-14.930	-51.406	19.091	1.000	9.02
ATOM	371	O	ILE	49	-15.531	-52.013	19.983	1.000	15.82
ATOM	372	N	GLU	50	-15.000	-50.085	18.932	1.000	5.34
ATOM	373	CA	GLU	50	-15.730	-49.277	19.911	1.000	12.03
ATOM	374	CB	GLU	50	-14.967	-47.984	20.222	1.000	10.36
ATOM	375	CG	GLU	50	-13.623	-48.203	20.889	1.000	7.32
ATOM	376	CD	GLU	50	-12.768	-46.966	21.056	1.000	7.06
ATOM	377	OE1	GLU	50	-12.744	-46.077	20.177	1.000	5.78
ATOM	378	OE2	GLU	50	-12.079	-46.870	22.101	1.000	25.19
ATOM	379	C	GLU	50	-17.145	-48.962	19.446	1.000	6.79
ATOM	380	O	GLU	50	-17.358	-48.318	18.423	1.000	8.80
ATOM	381	N	GLU	51	-18.118	-49.429	20.225	1.000	9.34
ATOM	382	CA	GLU	51	-19.524	-49.179	19.924	1.000	16.23
ATOM	383	CB	GLU	51	-20.173	-50.400	19.270	1.000	15.22
ATOM	384	CG	GLU	51	-19.757	-50.596	17.820	1.000	18.39
ATOM	385	CD	GLU	51	-20.348	-49.531	16.917	1.000	17.99
ATOM	386	OE1	GLU	51	-21.352	-48.912	17.332	1.000	26.29
ATOM	387	OE2	GLU	51	-19.820	-49.309	15.809	1.000	15.93
ATOM	388	C	GLU	51	-20.295	-48.788	21.184	1.000	10.51
ATOM	389	O	GLU	51	-21.202	-49.495	21.623	1.000	7.29
ATOM	390	N	GLY	52	-19.906	-47.655	21.751	1.000	5.90
ATOM	391	CA	GLY	52	-20.533	-47.140	22.961	1.000	3.93
ATOM	392	C	GLY	52	-21.329	-45.887	22.635	1.000	6.21
ATOM	393	O	GLY	52	-20.785	-44.950	22.057	1.000	16.40
ATOM	394	N	LEU	53	-22.607	-45.890	22.989	1.000	11.68
ATOM	395	CA	LEU	53	-23.498	-44.764	22.710	1.000	7.60
ATOM	396	CB	LEU	53	-24.627	-45.195	21.792	1.000	4.45
ATOM	397	CG	LEU	53	-25.576	-44.164	21.185	1.000	3.84
ATOM	398	CD1	LEU	53	-26.721	-43.872	22.141	1.000	15.09
ATOM	399	CD2	LEU	53	-24.856	-42.874	20.817	1.000	3.41
ATOM	400	C	LEU	53	-24.035	-44.204	24.023	1.000	5.05
ATOM	401	O	LEU	53	-24.664	-44.920	24.801	1.000	5.74
ATOM	402	N	SER	54	-23.771	-42.918	24.251	1.000	9.85
ATOM	403	CA	SER	54	-24.192	-42.296	25.502	1.000	10.24

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ATOM	404	CB	SER	54	-23.797	-40.819	25.524	1.000	7.63
ATOM	405	OG	SER	54	-22.395	-40.683	25.640	1.000	4.65
ATOM	406	C	SER	54	-25.695	-42.448	25.691	1.000	7.74
ATOM	407	O	SER	54	-26.438	-42.326	24.717	1.000	10.39
ATOM	408	N	ALA	55	-26.127	-42.713	26.920	1.000	0.00
ATOM	409	CA	ALA	55	-27.554	-42.749	27.218	1.000	0.00
ATOM	410	CB	ALA	55	-28.209	-41.474	26.713	1.000	0.00
ATOM	411	C	ALA	55	-28.235	-43.982	26.640	1.000	6.11
ATOM	412	O	ALA	55	-29.442	-44.179	26.816	1.000	2.57
ATOM	413	N	ARG	56	-27.474	-44.843	25.971	1.000	8.50
ATOM	414	CA	ARG	56	-27.997	-46.084	25.433	1.000	5.94
ATOM	415	CB	ARG	56	-26.919	-46.868	24.672	1.000	0.00
ATOM	416	CG	ARG	56	-27.420	-48.244	24.247	1.000	2.73
ATOM	417	CD	ARG	56	-26.467	-48.951	23.307	1.000	0.00
ATOM	418	NE	ARG	56	-26.552	-48.440	21.935	1.000	6.44
ATOM	419	CZ	ARG	56	-25.465	-48.325	21.170	1.000	11.18
ATOM	420	NH1	ARG	56	-24.283	-48.678	21.666	1.000	0.00
ATOM	421	NH2	ARG	56	-25.549	-47.861	19.928	1.000	1.13
ATOM	422	C	ARG	56	-28.539	-47.009	26.526	1.000	12.43
ATOM	423	O	ARG	56	-27.886	-47.179	27.556	1.000	10.16
ATOM	424	N	THR	57	-29.697	-47.592	26.262	1.000	9.24
ATOM	425	CA	THR	57	-30.376	-48.548	27.120	1.000	9.36
ATOM	426	CB	THR	57	-31.855	-48.161	27.315	1.000	4.78
ATOM	427	OG1	THR	57	-32.608	-48.509	26.146	1.000	3.70
ATOM	428	CG2	THR	57	-31.992	-46.656	27.484	1.000	0.00
ATOM	429	C	THR	57	-30.284	-49.953	26.532	1.000	10.18
ATOM	430	O	THR	57	-29.873	-50.099	25.378	1.000	12.60
ATOM	431	N	THR	58	-30.648	-50.987	27.286	1.000	5.87
ATOM	432	CA	THR	58	-30.574	-52.349	26.769	1.000	1.65
ATOM	433	CB	THR	58	-30.850	-53.410	27.853	1.000	5.35
ATOM	434	OG1	THR	58	-32.151	-53.196	28.413	1.000	12.48
ATOM	435	CG2	THR	58	-29.859	-53.311	29.002	1.000	11.47
ATOM	436	C	THR	58	-31.556	-52.569	25.624	1.000	1.31
ATOM	437	O	THR	58	-31.162	-52.902	24.506	1.000	7.78
ATOM	438	N	ASN	59	-32.856	-52.404	25.867	1.000	4.91
ATOM	439	CA	ASN	59	-33.810	-52.604	24.772	1.000	11.25
ATOM	440	CB	ASN	59	-34.150	-54.090	24.624	1.000	9.19
ATOM	441	CG	ASN	59	-35.186	-54.548	25.629	1.000	9.50
ATOM	442	OD1	ASN	59	-35.293	-54.000	26.725	1.000	13.36
ATOM	443	ND2	ASN	59	-35.965	-55.556	25.263	1.000	4.31
ATOM	444	C	ASN	59	-35.070	-51.775	24.960	1.000	8.67
ATOM	445	O	ASN	59	-36.172	-52.160	24.574	1.000	12.75
ATOM	446	N	ILE	60	-34.938	-50.587	25.548	1.000	10.46
ATOM	447	CA	ILE	60	-36.128	-49.752	25.722	1.000	10.70
ATOM	448	CB	ILE	60	-36.572	-49.721	27.198	1.000	11.36
ATOM	449	CG2	ILE	60	-35.465	-49.223	28.112	1.000	0.00
ATOM	450	CG1	ILE	60	-37.872	-48.940	27.417	1.000	8.05

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ATOM    451  CD1  ILE    60    -38.291 -48.800  28.860 1.000 27.90
ATOM    452  C    ILE    60    -35.879 -48.350  25.177 1.000 16.37
ATOM    453  O    ILE    60    -34.813 -47.773  25.374 1.000 28.53
ATOM    454  N    ASP    61    -36.861 -47.811  24.470 1.000 18.37
ATOM    455  CA   ASP    61    -36.838 -46.520  23.821 1.000 12.62
ATOM    456  CB   ASP    61    -38.110 -46.353  22.977 1.000 12.58
ATOM    457  CG   ASP    61    -38.111 -47.199  21.725 1.000 12.09
ATOM    458  OD1  ASP    61    -37.044 -47.723  21.349 1.000 16.37
ATOM    459  OD2  ASP    61    -39.197 -47.332  21.122 1.000 23.20
ATOM    460  C    ASP    61    -36.796 -45.350  24.794 1.000 11.54
ATOM    461  O    ASP    61    -37.626 -45.279  25.702 1.000  8.66
ATOM    462  N    ASP    62    -35.860 -44.428  24.603 1.000  8.03
ATOM    463  CA   ASP    62    -35.844 -43.228  25.431 1.000 14.39
ATOM    464  CB   ASP    62    -34.430 -42.656  25.565 1.000 13.94
ATOM    465  CG   ASP    62    -34.384 -41.598  26.656 1.000 18.06
ATOM    466  OD1  ASP    62    -33.609 -41.768  27.622 1.000 13.05
ATOM    467  OD2  ASP    62    -35.129 -40.604  26.536 1.000 20.19
ATOM    468  C    ASP    62    -36.759 -42.162  24.844 1.000 13.14
ATOM    469  O    ASP    62    -36.506 -41.698  23.731 1.000 14.36
ATOM    470  N    PRO    63    -37.800 -41.751  25.553 1.000  8.49
ATOM    471  CD   PRO    63    -38.102 -42.088  26.951 1.000  4.73
ATOM    472  CA   PRO    63    -38.805 -40.853  24.972 1.000 16.60
ATOM    473  CB   PRO    63    -39.802 -40.646  26.123 1.000 11.61
ATOM    474  CG   PRO    63    -39.020 -40.960  27.352 1.000  8.04
ATOM    475  C    PRO    63    -38.251 -39.504  24.531 1.000 19.70
ATOM    476  O    PRO    63    -38.924 -38.738  23.835 1.000 10.26
ATOM    477  N    THR    64    -37.024 -39.180  24.922 1.000 22.29
ATOM    478  CA   THR    64    -36.429 -37.908  24.534 1.000 19.30
ATOM    479  CB   THR    64    -35.852 -37.191  25.769 1.000 20.62
ATOM    480  OG1  THR    64    -34.550 -37.713  26.045 1.000 30.42
ATOM    481  CG2  THR    64    -36.718 -37.467  26.992 1.000  7.89
ATOM    482  C    THR    64    -35.329 -38.087  23.497 1.000 19.22
ATOM    483  O    THR    64    -34.609 -37.132  23.183 1.000 11.15
ATOM    484  N    ASP    65    -35.189 -39.301  22.965 1.000 15.61
ATOM    485  CA   ASP    65    -34.139 -39.542  21.967 1.000 18.78
ATOM    486  CB   ASP    65    -32.777 -39.286  22.605 1.000 20.50
ATOM    487  CG   ASP    65    -31.613 -39.348  21.638 1.000 17.33
ATOM    488  OD1  ASP    65    -31.767 -39.935  20.550 1.000 19.33
ATOM    489  OD2  ASP    65    -30.538 -38.810  21.983 1.000 15.26
ATOM    490  C    ASP    65    -34.241 -40.945  21.382 1.000 14.84
ATOM    491  O    ASP    65    -33.982 -41.936  22.060 1.000  8.38
ATOM    492  N    PRO    66    -34.638 -41.026  20.115 1.000 15.75
ATOM    493  CD   PRO    66    -34.896 -39.870  19.235 1.000 23.61
ATOM    494  CA   PRO    66    -34.882 -42.301  19.441 1.000  9.14
ATOM    495  CB   PRO    66    -35.693 -41.871  18.206 1.000 14.38
ATOM    496  CG   PRO    66    -35.210 -40.494  17.902 1.000 16.45
ATOM    497  C    PRO    66    -33.621 -43.029  18.995 1.000  8.15
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ATOM	498	O	PRO	66	-33.695	-44.041	18.283	1.000	12.38
ATOM	499	N	ARG	67	-32.446	-42.557	19.404	1.000	11.98
ATOM	500	CA	ARG	67	-31.209	-43.225	19.020	1.000	7.77
ATOM	501	CB	ARG	67	-30.081	-42.211	18.831	1.000	8.16
ATOM	502	CG	ARG	67	-30.162	-41.308	17.614	1.000	7.27
ATOM	503	CD	ARG	67	-29.078	-40.228	17.713	1.000	11.05
ATOM	504	NE	ARG	67	-29.378	-39.266	18.769	1.000	11.17
ATOM	505	CZ	ARG	67	-28.768	-38.115	19.001	1.000	13.35
ATOM	506	NH1	ARG	67	-27.756	-37.708	18.245	1.000	3.80
ATOM	507	NH2	ARG	67	-29.168	-37.347	20.010	1.000	9.93
ATOM	508	C	ARG	67	-30.728	-44.239	20.048	1.000	8.92
ATOM	509	O	ARG	67	-29.714	-44.887	19.774	1.000	13.65
ATOM	510	N	LEU	68	-31.389	-44.365	21.191	1.000	9.14
ATOM	511	CA	LEU	68	-30.805	-45.057	22.335	1.000	13.92
ATOM	512	CB	LEU	68	-31.052	-44.223	23.608	1.000	7.80
ATOM	513	CG	LEU	68	-30.899	-42.707	23.481	1.000	8.78
ATOM	514	CD1	LEU	68	-31.285	-41.987	24.770	1.000	13.12
ATOM	515	CD2	LEU	68	-29.477	-42.333	23.090	1.000	3.77
ATOM	516	C	LEU	68	-31.299	-46.478	22.571	1.000	16.19
ATOM	517	O	LEU	68	-30.895	-47.092	23.574	1.000	5.21
ATOM	518	N	ASN	69	-32.139	-47.056	21.716	1.000	7.75
ATOM	519	CA	ASN	69	-32.520	-48.457	21.927	1.000	6.53
ATOM	520	CB	ASN	69	-33.807	-48.842	21.198	1.000	6.25
ATOM	521	CG	ASN	69	-34.377	-50.172	21.658	1.000	11.70
ATOM	522	OD1	ASN	69	-33.732	-51.219	21.664	1.000	2.64
ATOM	523	ND2	ASN	69	-35.646	-50.164	22.057	1.000	10.84
ATOM	524	C	ASN	69	-31.406	-49.404	21.480	1.000	8.62
ATOM	525	O	ASN	69	-31.204	-49.617	20.287	1.000	14.61
ATOM	526	N	GLY	70	-30.697	-49.972	22.452	1.000	8.79
ATOM	527	CA	GLY	70	-29.582	-50.854	22.212	1.000	1.64
ATOM	528	C	GLY	70	-29.911	-52.031	21.316	1.000	6.17
ATOM	529	O	GLY	70	-29.189	-52.293	20.355	1.000	12.06
ATOM	530	N	ALA	71	-30.982	-52.744	21.622	1.000	1.39
ATOM	531	CA	ALA	71	-31.442	-53.885	20.843	1.000	5.92
ATOM	532	CB	ALA	71	-32.688	-54.457	21.529	1.000	3.81
ATOM	533	C	ALA	71	-31.766	-53.565	19.392	1.000	4.67
ATOM	534	O	ALA	71	-31.565	-54.391	18.490	1.000	0.00
ATOM	535	N	SER	72	-32.295	-52.371	19.121	1.000	3.88
ATOM	536	CA	SER	72	-32.687	-52.033	17.752	1.000	6.33
ATOM	537	CB	SER	72	-33.678	-50.870	17.759	1.000	4.05
ATOM	538	OG	SER	72	-33.023	-49.637	18.004	1.000	25.62
ATOM	539	C	SER	72	-31.468	-51.730	16.884	1.000	7.90
ATOM	540	O	SER	72	-31.568	-51.720	15.658	1.000	12.06
ATOM	541	N	TYR	73	-30.315	-51.505	17.498	1.000	8.51
ATOM	542	CA	TYR	73	-29.070	-51.210	16.789	1.000	8.77
ATOM	543	CB	TYR	73	-28.394	-50.029	17.478	1.000	10.31
ATOM	544	CG	TYR	73	-27.124	-49.453	16.913	1.000	11.92

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ATOM    545  CD1 TYR     73      -27.113 -48.329  16.090 1.000  8.49
ATOM    546  CE1 TYR     73      -25.931 -47.812  15.586 1.000  1.47
ATOM    547  CD2 TYR     73      -25.888 -50.018  17.201 1.000 10.36
ATOM    548  CE2 TYR     73      -24.704 -49.512  16.703 1.000  9.07
ATOM    549  CZ  TYR     73      -24.727 -48.398  15.890 1.000  5.36
ATOM    550  OH  TYR     73      -23.544 -47.902  15.391 1.000 10.80
ATOM    551  C   TYR     73      -28.148 -52.419  16.730 1.000 13.31
ATOM    552  O   TYR     73      -27.404 -52.630  15.764 1.000 10.40
ATOM    553  N   LEU     74      -28.172 -53.261  17.759 1.000  8.99
ATOM    554  CA  LEU     74      -27.204 -54.342  17.901 1.000  7.76
ATOM    555  CB  LEU     74      -27.554 -55.155  19.155 1.000  9.47
ATOM    556  CG  LEU     74      -26.402 -55.532  20.080 1.000 10.36
ATOM    557  CD1 LEU     74      -26.786 -56.729  20.939 1.000 25.33
ATOM    558  CD2 LEU     74      -25.137 -55.819  19.288 1.000 13.92
ATOM    559  C   LEU     74      -27.088 -55.253  16.687 1.000  5.72
ATOM    560  O   LEU     74      -25.980 -55.383  16.141 1.000  7.01
ATOM    561  N   PRO     75      -28.141 -55.907  16.219 1.000  6.99
ATOM    562  CD  PRO     75      -29.553 -55.794  16.615 1.000  1.55
ATOM    563  CA  PRO     75      -27.965 -56.896  15.140 1.000  7.57
ATOM    564  CB  PRO     75      -29.384 -57.401  14.855 1.000  5.01
ATOM    565  CG  PRO     75      -30.158 -57.063  16.086 1.000  6.27
ATOM    566  C   PRO     75      -27.364 -56.285  13.882 1.000  4.16
ATOM    567  O   PRO     75      -26.651 -56.971  13.158 1.000  4.35
ATOM    568  N   SER     76      -27.640 -55.014  13.615 1.000  6.22
ATOM    569  CA  SER     76      -27.050 -54.322  12.473 1.000  0.00
ATOM    570  CB  SER     76      -27.758 -52.978  12.261 1.000  0.00
ATOM    571  OG  SER     76      -29.120 -53.249  11.920 1.000  0.00
ATOM    572  C   SER     76      -25.554 -54.127  12.674 1.000  0.69
ATOM    573  O   SER     76      -24.767 -54.280  11.740 1.000  4.06
ATOM    574  N   CYS     77      -25.202 -53.802  13.911 1.000  2.82
ATOM    575  CA  CYS     77      -23.851 -53.599  14.384 1.000  2.99
ATOM    576  CB  CYS     77      -23.878 -53.202  15.868 1.000  0.00
ATOM    577  SG  CYS     77      -22.325 -52.508  16.451 1.000  8.78
ATOM    578  C   CYS     77      -22.962 -54.831  14.225 1.000 13.77
ATOM    579  O   CYS     77      -21.828 -54.700  13.755 1.000 12.12
ATOM    580  N   LEU     78      -23.455 -55.996  14.621 1.000 15.71
ATOM    581  CA  LEU     78      -22.751 -57.268  14.538 1.000 10.13
ATOM    582  CB  LEU     78      -23.617 -58.387  15.129 1.000  2.73
ATOM    583  CG  LEU     78      -23.777 -58.354  16.651 1.000  7.98
ATOM    584  CD1 LEU     78      -24.866 -59.319  17.085 1.000  3.36
ATOM    585  CD2 LEU     78      -22.451 -58.676  17.330 1.000  8.53
ATOM    586  C   LEU     78      -22.385 -57.650  13.106 1.000  9.88
ATOM    587  O   LEU     78      -21.222 -57.855  12.761 1.000 12.55
ATOM    588  N   ALA     79      -23.407 -57.748  12.271 1.000 11.93
ATOM    589  CA  ALA     79      -23.297 -58.022  10.848 1.000  2.98
ATOM    590  CB  ALA     79      -24.699 -58.042  10.255 1.000  0.32
ATOM    591  C   ALA     79      -22.393 -57.026  10.127 1.000  7.73
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ATOM	592	O	ALA	79	-21.724	-57.408	9.163	1.000	13.15
ATOM	593	N	THR	80	-22.337	-55.774	10.560	1.000	10.93
ATOM	594	CA	THR	80	-21.427	-54.757	10.044	1.000	6.56
ATOM	595	CB	THR	80	-21.703	-53.373	10.669	1.000	9.10
ATOM	596	OG1	THR	80	-23.013	-52.897	10.320	1.000	4.47
ATOM	597	CG2	THR	80	-20.722	-52.328	10.148	1.000	8.02
ATOM	598	C	THR	80	-19.970	-55.117	10.317	1.000	10.87
ATOM	599	O	THR	80	-19.103	-55.052	9.450	1.000	12.66
ATOM	600	N	HIS	81	-19.659	-55.512	11.548	1.000	13.90
ATOM	601	CA	HIS	81	-18.282	-55.720	11.978	1.000	13.04
ATOM	602	CB	HIS	81	-18.119	-55.195	13.418	1.000	15.15
ATOM	603	CG	HIS	81	-18.279	-53.704	13.502	1.000	10.10
ATOM	604	CD2	HIS	81	-19.202	-52.927	14.111	1.000	6.25
ATOM	605	ND1	HIS	81	-17.404	-52.833	12.889	1.000	7.20
ATOM	606	CE1	HIS	81	-17.775	-51.589	13.117	1.000	7.73
ATOM	607	NE2	HIS	81	-18.867	-51.616	13.863	1.000	6.24
ATOM	608	C	HIS	81	-17.827	-57.166	11.896	1.000	9.61
ATOM	609	O	HIS	81	-16.674	-57.460	12.216	1.000	10.35
ATOM	610	N	LEU	82	-18.689	-58.081	11.470	1.000	4.74
ATOM	611	CA	LEU	82	-18.257	-59.461	11.247	1.000	6.06
ATOM	612	CB	LEU	82	-19.399	-60.263	10.631	1.000	6.90
ATOM	613	CG	LEU	82	-20.535	-60.716	11.541	1.000	6.83
ATOM	614	CD1	LEU	82	-21.388	-61.774	10.851	1.000	11.79
ATOM	615	CD2	LEU	82	-19.987	-61.246	12.856	1.000	23.45
ATOM	616	C	LEU	82	-17.042	-59.500	10.337	1.000	6.51
ATOM	617	O	LEU	82	-16.972	-58.722	9.375	1.000	1.45
ATOM	618	N	PRO	83	-16.056	-60.360	10.556	1.000	7.15
ATOM	619	CD	PRO	83	-14.823	-60.374	9.731	1.000	0.00
ATOM	620	CA	PRO	83	-16.043	-61.394	11.583	1.000	5.44
ATOM	621	CB	PRO	83	-14.941	-62.341	11.067	1.000	9.33
ATOM	622	CG	PRO	83	-13.968	-61.405	10.415	1.000	7.09
ATOM	623	C	PRO	83	-15.638	-60.922	12.973	1.000	10.31
ATOM	624	O	PRO	83	-14.716	-60.125	13.110	1.000	16.21
ATOM	625	N	LEU	84	-16.319	-61.434	13.994	1.000	14.34
ATOM	626	CA	LEU	84	-16.009	-61.132	15.382	1.000	10.66
ATOM	627	CB	LEU	84	-17.165	-60.373	16.049	1.000	7.23
ATOM	628	CG	LEU	84	-17.485	-59.010	15.434	1.000	2.01
ATOM	629	CD1	LEU	84	-18.843	-58.518	15.902	1.000	8.19
ATOM	630	CD2	LEU	84	-16.382	-58.019	15.766	1.000	5.93
ATOM	631	C	LEU	84	-15.734	-62.386	16.203	1.000	7.34
ATOM	632	O	LEU	84	-16.299	-63.447	15.945	1.000	8.40
ATOM	633	N	ASP	85	-14.879	-62.247	17.208	1.000	8.68
ATOM	634	CA	ASP	85	-14.607	-63.332	18.146	1.000	10.21
ATOM	635	CB	ASP	85	-13.093	-63.433	18.382	1.000	15.96
ATOM	636	CG	ASP	85	-12.338	-63.789	17.117	1.000	11.01
ATOM	637	OD1	ASP	85	-12.343	-64.975	16.727	1.000	9.49
ATOM	638	OD2	ASP	85	-11.739	-62.878	16.518	1.000	28.18

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ATOM    639  C   ASP   85     -15.313 -63.142  19.477 1.000  0.00
ATOM    640  O   ASP   85     -15.778 -64.067  20.137 1.000  5.48
ATOM    641  N   LEU   86     -15.414 -61.907  19.958 1.000  7.62
ATOM    642  CA  LEU   86     -16.080 -61.695  21.243 1.000  8.84
ATOM    643  CB  LEU   86     -15.085 -61.690  22.403 1.000 12.15
ATOM    644  CG  LEU   86     -15.655 -61.580  23.822 1.000 13.98
ATOM    645  CD1 LEU   86     -16.562 -62.757  24.151 1.000  7.12
ATOM    646  CD2 LEU   86     -14.535 -61.477  24.850 1.000 10.28
ATOM    647  C   LEU   86     -16.841 -60.374  21.221 1.000  6.69
ATOM    648  O   LEU   86     -16.327 -59.409  20.649 1.000  8.05
ATOM    649  N   VAL   87     -18.013 -60.361  21.842 1.000  4.26
ATOM    650  CA  VAL   87     -18.752 -59.127  22.049 1.000  2.21
ATOM    651  CB  VAL   87     -20.150 -59.126  21.413 1.000  8.44
ATOM    652  CG1 VAL   87     -20.848 -57.808  21.722 1.000  2.51
ATOM    653  CG2 VAL   87     -20.104 -59.352  19.911 1.000  0.00
ATOM    654  C   VAL   87     -18.893 -58.869  23.551 1.000  7.05
ATOM    655  O   VAL   87     -19.472 -59.660  24.289 1.000  5.76
ATOM    656  N   ILE   88     -18.351 -57.746  24.010 1.000  7.24
ATOM    657  CA  ILE   88     -18.499 -57.336  25.400 1.000  6.18
ATOM    658  CB  ILE   88     -17.233 -56.652  25.938 1.000  6.54
ATOM    659  CG2 ILE   88     -17.458 -56.098  27.333 1.000 11.40
ATOM    660  CG1 ILE   88     -16.001 -57.559  25.902 1.000  6.21
ATOM    661  CD1 ILE   88     -14.734 -56.856  26.339 1.000  7.20
ATOM    662  C   ILE   88     -19.693 -56.394  25.506 1.000  4.68
ATOM    663  O   ILE   88     -19.817 -55.458  24.716 1.000 10.14
ATOM    664  N   ILE   89     -20.574 -56.672  26.457 1.000  7.74
ATOM    665  CA  ILE   89     -21.765 -55.857  26.645 1.000 12.20
ATOM    666  CB  ILE   89     -23.052 -56.635  26.306 1.000 12.51
ATOM    667  CG2 ILE   89     -24.253 -55.703  26.339 1.000 11.52
ATOM    668  CG1 ILE   89     -22.981 -57.390  24.979 1.000  6.47
ATOM    669  CD1 ILE   89     -24.250 -58.111  24.597 1.000  8.71
ATOM    670  C   ILE   89     -21.861 -55.340  28.078 1.000 11.05
ATOM    671  O   ILE   89     -22.169 -56.106  28.989 1.000  3.02
ATOM    672  N   MET   90     -21.590 -54.049  28.236 1.000  7.01
ATOM    673  CA  MET   90     -21.808 -53.359  29.492 1.000 11.48
ATOM    674  CB  MET   90     -20.535 -52.721  30.043 1.000  9.27
ATOM    675  CG  MET   90     -20.756 -52.097  31.415 1.000 10.33
ATOM    676 XD   MET   90     -19.202 -51.706  32.246 1.000 17.92
ATOM    677  CE  MET   90     -18.544 -50.475  31.124 1.000 12.70
ATOM    678  C   MET   90     -22.872 -52.262  29.325 1.000 12.90
ATOM    679  O   MET   90     -22.524 -51.143  28.954 1.000  0.00
ATOM    680  N   LEU   91     -24.108 -52.639  29.604 1.000  8.70
ATOM    681  CA  LEU   91     -25.292 -51.802  29.511 1.000 10.58
ATOM    682  CB  LEU   91     -26.114 -52.105  28.254 1.000  9.42
ATOM    683  CG  LEU   91     -25.573 -51.564  26.932 1.000  4.10
ATOM    684  CD1 LEU   91     -26.427 -52.046  25.772 1.000  0.00
ATOM    685  CD2 LEU   91     -25.506 -50.044  26.961 1.000  2.02
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ATOM	686	C	LEU	91	-26.169	-52.031	30.734	1.000	2.21
ATOM	687	O	LEU	91	-25.989	-53.066	31.388	1.000	10.59
ATOM	688	N	GLY	92	-27.087	-51.117	31.025	1.000	4.69
ATOM	689	CA	GLY	92	-27.963	-51.321	32.172	1.000	7.16
ATOM	690	C	GLY	92	-28.189	-50.092	33.027	1.000	0.00
ATOM	691	O	GLY	92	-29.266	-49.924	33.603	1.000	8.09
ATOM	692	N	THR	93	-27.204	-49.219	33.133	1.000	0.16
ATOM	693	CA	THR	93	-27.241	-48.005	33.929	1.000	9.42
ATOM	694	CB	THR	93	-25.927	-47.205	33.768	1.000	17.05
ATOM	695	OG1	THR	93	-24.811	-48.063	34.024	1.000	26.81
ATOM	696	CG2	THR	93	-25.847	-46.068	34.778	1.000	0.34
ATOM	697	C	THR	93	-28.386	-47.075	33.551	1.000	9.26
ATOM	698	O	THR	93	-29.037	-46.491	34.419	1.000	14.18
ATOM	699	N	ASN	94	-28.614	-46.927	32.250	1.000	0.69
ATOM	700	CA	ASN	94	-29.609	-45.981	31.755	1.000	5.12
ATOM	701	CB	ASN	94	-29.333	-45.677	30.274	1.000	9.42
ATOM	702	CG	ASN	94	-27.990	-44.983	30.120	1.000	10.74
ATOM	703	OD1	ASN	94	-27.679	-44.062	30.873	1.000	21.66
ATOM	704	ND2	ASN	94	-27.175	-45.417	29.174	1.000	18.23
ATOM	705	C	ASN	94	-31.029	-46.481	31.986	1.000	5.80
ATOM	706	O	ASN	94	-31.889	-45.654	32.317	1.000	4.04
ATOM	707	N	ASP	95	-31.282	-47.777	31.863	1.000	4.02
ATOM	708	CA	ASP	95	-32.568	-48.411	32.137	1.000	7.86
ATOM	709	CB	ASP	95	-32.522	-49.913	31.880	1.000	5.49
ATOM	710	CG	ASP	95	-32.090	-50.392	30.521	1.000	10.09
ATOM	711	OD1	ASP	95	-30.998	-50.021	30.040	1.000	16.22
ATOM	712	OD2	ASP	95	-32.843	-51.184	29.907	1.000	15.98
ATOM	713	C	ASP	95	-33.020	-48.208	33.591	1.000	9.17
ATOM	714	O	ASP	95	-34.188	-48.361	33.958	1.000	0.43
ATOM	715	N	THR	96	-32.051	-47.882	34.421	1.000	11.45
ATOM	716	CA	THR	96	-32.122	-47.529	35.823	1.000	16.75
ATOM	717	CB	THR	96	-30.697	-47.638	36.412	1.000	24.78
ATOM	718	OG1	THR	96	-30.607	-48.784	37.274	1.000	17.62
ATOM	719	CG2	THR	96	-30.350	-46.409	37.229	1.000	12.12
ATOM	720	C	THR	96	-32.697	-46.132	35.997	1.000	12.12
ATOM	721	O	THR	96	-33.047	-45.678	37.088	1.000	10.94
ATOM	722	N	LYS	97	-32.820	-45.406	34.883	1.000	12.18
ATOM	723	CA	LYS	97	-33.387	-44.060	34.954	1.000	14.27
ATOM	724	CB	LYS	97	-33.247	-43.336	33.620	1.000	13.25
ATOM	725	CG	LYS	97	-31.996	-42.477	33.500	1.000	11.50
ATOM	726	CD	LYS	97	-31.819	-41.935	32.086	1.000	3.08
ATOM	727	CE	LYS	97	-30.344	-41.856	31.717	1.000	0.00
ATOM	728	NZ	LYS	97	-30.131	-41.152	30.416	1.000	0.00
ATOM	729	C	LYS	97	-34.848	-44.112	35.403	1.000	12.44
ATOM	730	O	LYS	97	-35.636	-44.914	34.911	1.000	8.04
ATOM	731	N	ALA	98	-35.179	-43.246	36.355	1.000	11.97
ATOM	732	CA	ALA	98	-36.454	-43.218	37.047	1.000	4.97

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ATOM	733	CB	ALA	98	-36.522	-41.982	37.943	1.000	3.36
ATOM	734	C	ALA	98	-37.641	-43.246	36.100	1.000	12.00
ATOM	735	O	ALA	98	-38.651	-43.905	36.355	1.000	22.61
ATOM	736	N	TYR	99	-37.535	-42.518	34.988	1.000	12.39
ATOM	737	CA	TYR	99	-38.695	-42.403	34.107	1.000	7.25
ATOM	738	CB	TYR	99	-38.521	-41.297	33.087	1.000	9.11
ATOM	739	CG	TYR	99	-37.300	-41.251	32.217	1.000	15.58
ATOM	740	CD1	TYR	99	-37.261	-41.912	30.995	1.000	13.09
ATOM	741	CE1	TYR	99	-36.144	-41.874	30.186	1.000	9.06
ATOM	742	CD2	TYR	99	-36.173	-40.533	32.598	1.000	14.48
ATOM	743	CE2	TYR	99	-35.051	-40.482	31.796	1.000	15.13
ATOM	744	CZ	TYR	99	-35.044	-41.154	30.591	1.000	11.74
ATOM	745	OH	TYR	99	-33.925	-41.102	29.794	1.000	6.20
ATOM	746	C	TYR	99	-38.990	-43.726	33.413	1.000	11.25
ATOM	747	O	TYR	99	-40.121	-43.927	32.963	1.000	12.89
ATOM	748	N	PHE	100	-37.993	-44.606	33.351	1.000	4.63
ATOM	749	CA	PHE	100	-38.237	-45.908	32.731	1.000	1.01
ATOM	750	CB	PHE	100	-36.903	-46.556	32.348	1.000	3.41
ATOM	751	CG	PHE	100	-36.316	-45.980	31.070	1.000	11.77
ATOM	752	CD1	PHE	100	-35.018	-45.506	31.032	1.000	7.50
ATOM	753	CD2	PHE	100	-37.080	-45.919	29.917	1.000	16.94
ATOM	754	CE1	PHE	100	-34.489	-44.981	29.868	1.000	7.31
ATOM	755	CE2	PHE	100	-36.557	-45.398	28.748	1.000	12.92
ATOM	756	CZ	PHE	100	-35.260	-44.925	28.722	1.000	7.58
ATOM	757	C	PHE	100	-39.051	-46.829	33.628	1.000	6.94
ATOM	758	O	PHE	100	-39.711	-47.750	33.131	1.000	9.31
ATOM	759	N	ARG	101	-39.032	-46.629	34.943	1.000	12.10
ATOM	760	CA	ARG	101	-39.783	-47.468	35.869	1.000	12.96
ATOM	761	CB	ARG	101	-41.294	-47.296	35.695	1.000	16.21
ATOM	762	CG	ARG	101	-41.890	-45.959	36.087	1.000	19.51
ATOM	763	CD	ARG	101	-43.376	-45.918	35.740	1.000	25.82
ATOM	764	NE	ARG	101	-43.818	-44.553	35.466	1.000	31.88
ATOM	765	CZ	ARG	101	-43.797	-43.583	36.373	1.000	33.97
ATOM	766	NH1	ARG	101	-43.355	-43.839	37.599	1.000	43.49
ATOM	767	NH2	ARG	101	-44.206	-42.361	36.067	1.000	44.85
ATOM	768	C	ARG	101	-39.472	-48.955	35.704	1.000	12.20
ATOM	769	O	ARG	101	-40.376	-49.782	35.878	1.000	12.48
ATOM	770	N	ARG	102	-38.238	-49.319	35.378	1.000	8.86
ATOM	771	CA	ARG	102	-37.887	-50.733	35.264	1.000	11.00
ATOM	772	CB	ARG	102	-36.899	-50.962	34.115	1.000	6.96
ATOM	773	CG	ARG	102	-37.497	-50.805	32.720	1.000	9.64
ATOM	774	CD	ARG	102	-36.518	-51.198	31.624	1.000	8.07
ATOM	775	NE	ARG	102	-37.140	-51.842	30.474	1.000	4.64
ATOM	776	CZ	ARG	102	-36.540	-52.606	29.571	1.000	7.34
ATOM	777	NH1	ARG	102	-35.240	-52.877	29.628	1.000	1.45
ATOM	778	NH2	ARG	102	-37.232	-53.131	28.567	1.000	6.11
ATOM	779	C	ARG	102	-37.320	-51.275	36.577	1.000	11.09

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ATOM	780	O	ARG	102	-36.734	-50.567	37.394	1.000	10.02
ATOM	781	N	THR	103	-37.497	-52.573	36.785	1.000	11.01
ATOM	782	CA	THR	103	-36.898	-53.307	37.893	1.000	12.65
ATOM	783	CB	THR	103	-37.844	-54.376	38.462	1.000	7.64
ATOM	784	OG1	THR	103	-38.083	-55.384	37.468	1.000	11.29
ATOM	785	CG2	THR	103	-39.199	-53.771	38.790	1.000	15.33
ATOM	786	C	THR	103	-35.618	-53.966	37.390	1.000	10.55
ATOM	787	O	THR	103	-35.409	-53.986	36.173	1.000	9.17
ATOM	788	N	PRO	104	-34.765	-54.474	38.264	1.000	10.17
ATOM	789	CD	PRO	104	-34.799	-54.363	39.731	1.000	14.03
ATOM	790	CA	PRO	104	-33.598	-55.230	37.803	1.000	6.81
ATOM	791	CB	PRO	104	-32.968	-55.748	39.094	1.000	5.25
ATOM	792	CG	PRO	104	-33.402	-54.759	40.129	1.000	8.07
ATOM	793	C	PRO	104	-34.010	-56.400	36.911	1.000	5.89
ATOM	794	O	PRO	104	-33.251	-56.728	35.998	1.000	5.49
ATOM	795	N	LEU	105	-35.164	-56.994	37.173	1.000	2.55
ATOM	796	CA	LEU	105	-35.690	-58.071	36.341	1.000	10.27
ATOM	797	CB	LEU	105	-36.989	-58.642	36.890	1.000	11.51
ATOM	798	CG	LEU	105	-37.304	-60.122	36.695	1.000	16.39
ATOM	799	CD1	LEU	105	-38.804	-60.319	36.480	1.000	4.05
ATOM	800	CD2	LEU	105	-36.533	-60.744	35.542	1.000	15.49
ATOM	801	C	LEU	105	-35.923	-57.566	34.915	1.000	14.30
ATOM	802	O	LEU	105	-35.415	-58.168	33.969	1.000	14.22
ATOM	803	N	ASP	106	-36.686	-56.484	34.791	1.000	11.11
ATOM	804	CA	ASP	106	-36.922	-55.878	33.482	1.000	8.08
ATOM	805	CB	ASP	106	-37.636	-54.538	33.621	1.000	14.02
ATOM	806	CG	ASP	106	-39.046	-54.638	34.152	1.000	13.88
ATOM	807	OD1	ASP	106	-39.726	-55.653	33.875	1.000	19.94
ATOM	808	OD2	ASP	106	-39.479	-53.686	34.843	1.000	4.29
ATOM	809	C	ASP	106	-35.607	-55.668	32.734	1.000	7.79
ATOM	810	O	ASP	106	-35.504	-55.987	31.554	1.000	10.52
ATOM	811	N	ILE	107	-34.614	-55.131	33.438	1.000	5.00
ATOM	812	CA	ILE	107	-33.321	-54.814	32.845	1.000	6.63
ATOM	813	CB	ILE	107	-32.444	-54.016	33.828	1.000	14.49
ATOM	814	CG2	ILE	107	-31.125	-53.622	33.184	1.000	7.24
ATOM	815	CG1	ILE	107	-33.146	-52.790	34.415	1.000	16.93
ATOM	816	CD1	ILE	107	-32.174	-51.779	34.992	1.000	19.38
ATOM	817	C	ILE	107	-32.564	-56.059	32.405	1.000	5.12
ATOM	818	O	ILE	107	-31.877	-56.024	31.381	1.000	4.80
ATOM	819	N	ALA	108	-32.691	-57.148	33.157	1.000	5.34
ATOM	820	CA	ALA	108	-32.021	-58.398	32.812	1.000	4.25
ATOM	821	CB	ALA	108	-32.089	-59.399	33.956	1.000	2.49
ATOM	822	C	ALA	108	-32.637	-59.018	31.568	1.000	2.89
ATOM	823	O	ALA	108	-31.952	-59.619	30.738	1.000	11.68
ATOM	824	N	LEU	109	-33.956	-58.864	31.449	1.000	0.00
ATOM	825	CA	LEU	109	-34.609	-59.401	30.251	1.000	6.18
ATOM	826	CB	LEU	109	-36.125	-59.391	30.435	1.000	12.37

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ATOM	827	CG	LEU	109	-36.674	-60.463	31.386	1.000	15.66
ATOM	828	CD1	LEU	109	-37.985	-60.004	32.001	1.000	27.44
ATOM	829	CD2	LEU	109	-36.854	-61.794	30.672	1.000	3.14
ATOM	830	C	LEU	109	-34.171	-58.620	29.022	1.000	10.30
ATOM	831	O	LEU	109	-34.035	-59.139	27.915	1.000	18.00
ATOM	832	N	GLY	110	-33.918	-57.323	29.193	1.000	11.78
ATOM	833	CA	GLY	110	-33.426	-56.535	28.069	1.000	8.26
ATOM	834	C	GLY	110	-32.028	-56.976	27.666	1.000	7.06
ATOM	835	O	GLY	110	-31.757	-57.155	26.482	1.000	18.68
ATOM	836	N	MET	111	-31.149	-57.149	28.651	1.000	5.04
ATOM	837	CA	MET	111	-29.812	-57.661	28.414	1.000	4.52
ATOM	838	CB	MET	111	-28.962	-57.717	29.683	1.000	1.61
ATOM	839	CG	MET	111	-27.663	-58.503	29.542	1.000	0.00
ATOM	840	XD	MET	111	-26.456	-57.694	28.453	1.000	16.83
ATOM	841	CE	MET	111	-25.895	-56.355	29.497	1.000	5.08
ATOM	842	C	MET	111	-29.915	-59.066	27.821	1.000	6.40
ATOM	843	O	MET	111	-29.098	-59.476	27.005	1.000	8.66
ATOM	844	N	SER	112	-30.937	-59.795	28.270	1.000	9.55
ATOM	845	CA	SER	112	-31.140	-61.133	27.731	1.000	8.05
ATOM	846	CB	SER	112	-32.322	-61.821	28.405	1.000	10.37
ATOM	847	OG	SER	112	-33.488	-61.744	27.609	1.000	8.11
ATOM	848	C	SER	112	-31.341	-61.034	26.217	1.000	6.07
ATOM	849	O	SER	112	-30.761	-61.823	25.471	1.000	9.26
ATOM	850	N	VAL	113	-32.142	-60.065	25.803	1.000	4.80
ATOM	851	CA	VAL	113	-32.424	-59.788	24.401	1.000	9.22
ATOM	852	CB	VAL	113	-33.414	-58.615	24.266	1.000	9.35
ATOM	853	CG1	VAL	113	-33.350	-57.979	22.886	1.000	0.53
ATOM	854	CG2	VAL	113	-34.830	-59.090	24.567	1.000	15.43
ATOM	855	C	VAL	113	-31.149	-59.490	23.616	1.000	18.19
ATOM	856	O	VAL	113	-31.027	-59.900	22.456	1.000	17.08
ATOM	857	N	LEU	114	-30.199	-58.791	24.235	1.000	16.22
ATOM	858	CA	LEU	114	-28.948	-58.431	23.570	1.000	9.05
ATOM	859	CB	LEU	114	-28.220	-57.329	24.341	1.000	4.93
ATOM	860	CG	LEU	114	-28.938	-55.983	24.427	1.000	6.23
ATOM	861	CD1	LEU	114	-28.122	-54.973	25.221	1.000	8.47
ATOM	862	CD2	LEU	114	-29.228	-55.450	23.032	1.000	0.00
ATOM	863	C	LEU	114	-28.018	-59.628	23.407	1.000	5.15
ATOM	864	O	LEU	114	-27.310	-59.762	22.410	1.000	8.05
ATOM	865	N	VAL	115	-28.028	-60.503	24.403	1.000	5.78
ATOM	866	CA	VAL	115	-27.223	-61.717	24.373	1.000	8.93
ATOM	867	CB	VAL	115	-27.202	-62.383	25.762	1.000	8.05
ATOM	868	CG1	VAL	115	-26.501	-63.729	25.720	1.000	0.00
ATOM	869	CG2	VAL	115	-26.543	-61.439	26.759	1.000	0.00
ATOM	870	C	VAL	115	-27.763	-62.685	23.330	1.000	9.50
ATOM	871	O	VAL	115	-27.007	-63.390	22.662	1.000	9.58
ATOM	872	N	THR	116	-29.087	-62.715	23.179	1.000	8.15
ATOM	873	CA	THR	116	-29.688	-63.617	22.199	1.000	8.38

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ATOM	874	CB	THR	116	-31.222	-63.622	22.327	1.000	12.50
ATOM	875	OG1	THR	116	-31.575	-64.207	23.585	1.000	13.40
ATOM	876	CG2	THR	116	-31.848	-64.479	21.233	1.000	10.82
ATOM	877	C	THR	116	-29.316	-63.241	20.771	1.000	5.56
ATOM	878	O	THR	116	-29.011	-64.127	19.966	1.000	5.27
ATOM	879	N	GLN	117	-29.345	-61.945	20.473	1.000	8.17
ATOM	880	CA	GLN	117	-28.956	-61.430	19.160	1.000	9.93
ATOM	881	CB	GLN	117	-29.166	-59.920	19.080	1.000	3.66
ATOM	882	CG	GLN	117	-30.592	-59.440	19.279	1.000	6.21
ATOM	883	CD	GLN	117	-30.699	-57.933	19.390	1.000	7.09
ATOM	884	OE1	GLN	117	-29.801	-57.260	19.896	1.000	12.85
ATOM	885	NE2	GLN	117	-31.811	-57.376	18.914	1.000	7.39
ATOM	886	C	GLN	117	-27.499	-61.761	18.847	1.000	11.60
ATOM	887	O	GLN	117	-27.105	-62.023	17.706	1.000	9.03
ATOM	888	N	VAL	118	-26.652	-61.751	19.879	1.000	11.77
ATOM	889	CA	VAL	118	-25.258	-62.146	19.659	1.000	8.34
ATOM	890	CB	VAL	118	-24.340	-61.768	20.831	1.000	0.49
ATOM	891	CG1	VAL	118	-22.892	-62.118	20.499	1.000	21.94
ATOM	892	CG2	VAL	118	-24.452	-60.291	21.169	1.000	3.31
ATOM	893	C	VAL	118	-25.166	-63.652	19.417	1.000	10.48
ATOM	894	O	VAL	118	-24.354	-64.107	18.607	1.000	10.54
ATOM	895	N	LEU	119	-25.993	-64.431	20.112	1.000	7.97
ATOM	896	CA	LEU	119	-25.916	-65.885	19.993	1.000	8.73
ATOM	897	CB	LEU	119	-26.679	-66.572	21.135	1.000	8.06
ATOM	898	CG	LEU	119	-25.981	-66.556	22.498	1.000	21.06
ATOM	899	CD1	LEU	119	-26.800	-67.296	23.548	1.000	5.53
ATOM	900	CD2	LEU	119	-24.580	-67.150	22.403	1.000	21.96
ATOM	901	C	LEU	119	-26.446	-66.362	18.649	1.000	5.78
ATOM	902	O	LEU	119	-26.022	-67.409	18.153	1.000	14.06
ATOM	903	N	THR	120	-27.364	-65.608	18.053	1.000	8.82
ATOM	904	CA	THR	120	-27.964	-65.985	16.780	1.000	0.00
ATOM	905	CB	THR	120	-29.497	-65.798	16.815	1.000	6.15
ATOM	906	OG1	THR	120	-29.805	-64.405	16.969	1.000	10.14
ATOM	907	CG2	THR	120	-30.121	-66.535	17.994	1.000	0.76
ATOM	908	C	THR	120	-27.419	-65.198	15.594	1.000	10.30
ATOM	909	O	THR	120	-28.061	-65.190	14.537	1.000	13.46
ATOM	910	N	SER	121	-26.272	-64.533	15.700	1.000	11.26
ATOM	911	CA	SER	121	-25.774	-63.675	14.636	1.000	7.70
ATOM	912	CB	SER	121	-25.000	-62.487	15.240	1.000	5.36
ATOM	913	OG	SER	121	-23.826	-62.954	15.886	1.000	3.70
ATOM	914	C	SER	121	-24.852	-64.353	13.629	1.000	7.89
ATOM	915	O	SER	121	-24.360	-63.660	12.730	1.000	13.24
ATOM	916	N	ALA	122	-24.603	-65.645	13.755	1.000	11.50
ATOM	917	CA	ALA	122	-23.748	-66.370	12.820	1.000	12.48
ATOM	918	CB	ALA	122	-23.820	-67.868	13.098	1.000	3.73
ATOM	919	C	ALA	122	-24.124	-66.083	11.370	1.000	7.92
ATOM	920	O	ALA	122	-25.311	-66.050	11.042	1.000	8.42

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ATOM	921	N	GLY	123	-23.125	-65.859	10.529	1.000	7.14
ATOM	922	CA	GLY	123	-23.316	-65.625	9.115	1.000	3.98
ATOM	923	C	GLY	123	-23.643	-64.196	8.735	1.000	12.34
ATOM	924	O	GLY	123	-23.445	-63.822	7.571	1.000	1.55
ATOM	925	N	GLY	124	-24.132	-63.404	9.683	1.000	19.09
ATOM	926	CA	GLY	124	-24.506	-62.016	9.471	1.000	13.26
ATOM	927	C	GLY	124	-25.277	-61.809	8.186	1.000	10.25
ATOM	928	O	GLY	124	-26.403	-62.278	8.018	1.000	10.97
ATOM	929	N	VAL	125	-24.684	-61.110	7.217	1.000	12.50
ATOM	930	CA	VAL	125	-25.365	-60.956	5.930	1.000	9.40
ATOM	931	CB	VAL	125	-25.557	-59.477	5.559	1.000	14.11
ATOM	932	CG1	VAL	125	-26.156	-59.326	4.168	1.000	13.51
ATOM	933	CG2	VAL	125	-26.455	-58.786	6.578	1.000	22.31
ATOM	934	C	VAL	125	-24.588	-61.675	4.833	1.000	6.71
ATOM	935	O	VAL	125	-23.580	-61.151	4.368	1.000	4.54
ATOM	936	N	GLY	126	-25.047	-62.850	4.427	1.000	14.20
ATOM	937	CA	GLY	126	-24.466	-63.654	3.377	1.000	9.15
ATOM	938	C	GLY	126	-23.012	-64.018	3.580	1.000	10.06
ATOM	939	O	GLY	126	-22.225	-64.068	2.629	1.000	4.29
ATOM	940	N	THR	127	-22.595	-64.295	4.811	1.000	6.29
ATOM	941	CA	THR	127	-21.214	-64.701	5.050	1.000	3.83
ATOM	942	CB	THR	127	-20.470	-63.707	5.957	1.000	8.35
ATOM	943	OG1	THR	127	-20.719	-64.001	7.339	1.000	16.55
ATOM	944	CG2	THR	127	-20.987	-62.295	5.716	1.000	11.34
ATOM	945	C	THR	127	-21.143	-66.099	5.663	1.000	1.10
ATOM	946	O	THR	127	-22.159	-66.699	6.001	1.000	4.52
ATOM	947	N	THR	128	-19.921	-66.590	5.790	1.000	9.21
ATOM	948	CA	THR	128	-19.546	-67.893	6.299	1.000	8.72
ATOM	949	CB	THR	128	-18.451	-68.505	5.397	1.000	10.99
ATOM	950	OG1	THR	128	-17.447	-67.497	5.236	1.000	7.85
ATOM	951	CG2	THR	128	-18.976	-68.853	4.015	1.000	3.45
ATOM	952	C	THR	128	-18.995	-67.821	7.718	1.000	13.03
ATOM	953	O	THR	128	-18.450	-68.788	8.255	1.000	8.50
ATOM	954	N	TYR	129	-19.127	-66.646	8.315	1.000	10.20
ATOM	955	CA	TYR	129	-18.542	-66.357	9.615	1.000	7.58
ATOM	956	CB	TYR	129	-18.323	-64.853	9.722	1.000	8.22
ATOM	957	CG	TYR	129	-17.246	-64.280	8.835	1.000	11.97
ATOM	958	CD1	TYR	129	-17.514	-63.176	8.031	1.000	8.62
ATOM	959	CE1	TYR	129	-16.547	-62.636	7.211	1.000	7.23
ATOM	960	CD2	TYR	129	-15.970	-64.827	8.799	1.000	12.10
ATOM	961	CE2	TYR	129	-14.991	-64.290	7.982	1.000	16.92
ATOM	962	CZ	TYR	129	-15.288	-63.196	7.193	1.000	16.10
ATOM	963	OH	TYR	129	-14.315	-62.655	6.383	1.000	11.56
ATOM	964	C	TYR	129	-19.416	-66.840	10.765	1.000	9.63
ATOM	965	O	TYR	129	-20.644	-66.723	10.714	1.000	13.75
ATOM	966	N	PRO	130	-18.789	-67.380	11.804	1.000	8.51
ATOM	967	CD	PRO	130	-17.336	-67.523	12.004	1.000	10.11

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ATOM	968	CA	PRO	130	-19.549	-67.914	12.938	1.000	5.53
ATOM	969	CB	PRO	130	-18.522	-68.804	13.647	1.000	8.51
ATOM	970	CG	PRO	130	-17.227	-68.097	13.397	1.000	11.17
ATOM	971	C	PRO	130	-19.983	-66.791	13.872	1.000	7.77
ATOM	972	O	PRO	130	-19.500	-65.667	13.730	1.000	2.72
ATOM	973	N	ALA	131	-20.873	-67.117	14.799	1.000	7.61
ATOM	974	CA	ALA	131	-21.305	-66.205	15.844	1.000	2.73
ATOM	975	CB	ALA	131	-22.537	-66.747	16.554	1.000	0.00
ATOM	976	C	ALA	131	-20.174	-65.984	16.842	1.000	8.30
ATOM	977	O	ALA	131	-19.502	-66.942	17.223	1.000	12.18
ATOM	978	N	PRO	132	-19.937	-64.752	17.273	1.000	14.28
ATOM	979	CD	PRO	132	-20.610	-63.516	16.842	1.000	11.04
ATOM	980	CA	PRO	132	-18.901	-64.505	18.284	1.000	12.37
ATOM	981	CB	PRO	132	-18.696	-62.992	18.181	1.000	14.35
ATOM	982	CG	PRO	132	-20.032	-62.472	17.753	1.000	12.70
ATOM	983	C	PRO	132	-19.395	-64.884	19.675	1.000	12.80
ATOM	984	O	PRO	132	-20.608	-65.027	19.856	1.000	21.24
ATOM	985	N	LYS	133	-18.497	-65.051	20.641	1.000	14.17
ATOM	986	CA	LYS	133	-18.903	-65.337	22.017	1.000	14.31
ATOM	987	CB	LYS	133	-17.760	-65.881	22.869	1.000	14.22
ATOM	988	CG	LYS	133	-17.050	-67.101	22.317	1.000	13.51
ATOM	989	CD	LYS	133	-15.746	-67.358	23.057	1.000	18.76
ATOM	990	CE	LYS	133	-15.463	-68.849	23.174	1.000	21.23
ATOM	991	NZ	LYS	133	-15.154	-69.237	24.580	1.000	37.08
ATOM	992	C	LYS	133	-19.441	-64.066	22.667	1.000	10.23
ATOM	993	O	LYS	133	-19.319	-62.982	22.091	1.000	4.45
ATOM	994	N	VAL	134	-20.032	-64.194	23.853	1.000	4.74
ATOM	995	CA	VAL	134	-20.562	-63.000	24.507	1.000	10.55
ATOM	996	CB	VAL	134	-22.106	-62.964	24.490	1.000	11.86
ATOM	997	CG1	VAL	134	-22.586	-61.523	24.423	1.000	0.00
ATOM	998	CG2	VAL	134	-22.659	-63.778	23.334	1.000	29.88
ATOM	999	C	VAL	134	-20.129	-62.885	25.963	1.000	12.01
ATOM	1000	O	VAL	134	-20.215	-63.837	26.736	1.000	27.94
ATOM	1001	N	LEU	135	-19.676	-61.703	26.357	1.000	12.21
ATOM	1002	CA	LEU	135	-19.364	-61.443	27.757	1.000	14.41
ATOM	1003	CB	LEU	135	-17.975	-60.835	27.898	1.000	17.37
ATOM	1004	CG	LEU	135	-17.123	-61.223	29.105	1.000	18.57
ATOM	1005	CD1	LEU	135	-15.993	-60.213	29.264	1.000	4.42
ATOM	1006	CD2	LEU	135	-17.932	-61.341	30.387	1.000	6.01
ATOM	1007	C	LEU	135	-20.397	-60.497	28.360	1.000	17.03
ATOM	1008	O	LEU	135	-20.485	-59.326	27.984	1.000	14.19
ATOM	1009	N	VAL	136	-21.196	-60.988	29.303	1.000	19.10
ATOM	1010	CA	VAL	136	-22.167	-60.110	29.954	1.000	14.45
ATOM	1011	CB	VAL	136	-23.344	-60.925	30.511	1.000	13.65
ATOM	1012	CG1	VAL	136	-24.272	-60.045	31.335	1.000	8.06
ATOM	1013	CG2	VAL	136	-24.080	-61.596	29.362	1.000	0.00
ATOM	1014	C	VAL	136	-21.498	-59.327	31.073	1.000	10.63

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ATOM	1015	O	VAL	136	-20.929	-59.948	31.971	1.000	7.12
ATOM	1016	N	VAL	137	-21.556	-57.997	31.027	1.000	7.93
ATOM	1017	CA	VAL	137	-20.882	-57.215	32.056	1.000	6.63
ATOM	1018	CB	VAL	137	-19.699	-56.397	31.497	1.000	6.08
ATOM	1019	CG1	VAL	137	-19.115	-55.512	32.595	1.000	6.59
ATOM	1020	CG2	VAL	137	-18.609	-57.291	30.936	1.000	10.34
ATOM	1021	C	VAL	137	-21.828	-56.255	32.775	1.000	6.02
ATOM	1022	O	VAL	137	-22.319	-55.273	32.219	1.000	11.10
ATOM	1023	N	SER	138	-22.061	-56.558	34.040	1.000	6.05
ATOM	1024	CA	SER	138	-22.800	-55.715	34.972	1.000	9.77
ATOM	1025	CB	SER	138	-23.139	-56.523	36.223	1.000	16.98
ATOM	1026	OG	SER	138	-23.850	-55.804	37.202	1.000	19.18
ATOM	1027	C	SER	138	-21.944	-54.496	35.276	1.000	8.41
ATOM	1028	O	SER	138	-20.779	-54.646	35.652	1.000	13.52
ATOM	1029	N	PRO	139	-22.459	-53.287	35.096	1.000	12.22
ATOM	1030	CD	PRO	139	-23.803	-52.952	34.599	1.000	11.54
ATOM	1031	CA	PRO	139	-21.657	-52.087	35.389	1.000	6.14
ATOM	1032	CB	PRO	139	-22.422	-51.015	34.608	1.000	7.78
ATOM	1033	CG	PRO	139	-23.848	-51.455	34.731	1.000	3.74
ATOM	1034	C	PRO	139	-21.620	-51.775	36.875	1.000	3.92
ATOM	1035	O	PRO	139	-22.460	-52.217	37.664	1.000	10.47
ATOM	1036	N	PRO	140	-20.636	-51.014	37.347	1.000	8.52
ATOM	1037	CD	PRO	140	-19.524	-50.412	36.611	1.000	3.33
ATOM	1038	CA	PRO	140	-20.591	-50.724	38.788	1.000	13.50
ATOM	1039	CB	PRO	140	-19.251	-50.012	38.971	1.000	12.27
ATOM	1040	CG	PRO	140	-18.843	-49.543	37.623	1.000	6.73
ATOM	1041	C	PRO	140	-21.748	-49.832	39.228	1.000	15.77
ATOM	1042	O	PRO	140	-22.321	-49.073	38.445	1.000	21.96
ATOM	1043	N	PRO	141	-22.103	-49.939	40.505	1.000	4.93
ATOM	1044	CD	PRO	141	-21.487	-50.799	41.528	1.000	0.26
ATOM	1045	CA	PRO	141	-23.230	-49.172	41.036	1.000	3.17
ATOM	1046	CB	PRO	141	-23.254	-49.560	42.521	1.000	4.18
ATOM	1047	CG	PRO	141	-22.591	-50.897	42.556	1.000	0.00
ATOM	1048	C	PRO	141	-23.014	-47.671	40.890	1.000	10.32
ATOM	1049	O	PRO	141	-21.876	-47.203	40.900	1.000	17.58
ATOM	1050	N	LEU	142	-24.120	-46.942	40.760	1.000	9.20
ATOM	1051	CA	LEU	142	-24.079	-45.490	40.729	1.000	7.44
ATOM	1052	CB	LEU	142	-25.421	-44.900	40.288	1.000	7.55
ATOM	1053	CG	LEU	142	-25.775	-45.119	38.812	1.000	13.23
ATOM	1054	CD1	LEU	142	-27.262	-44.901	38.566	1.000	0.00
ATOM	1055	CD2	LEU	142	-24.932	-44.218	37.921	1.000	1.85
ATOM	1056	C	LEU	142	-23.711	-44.945	42.109	1.000	13.38
ATOM	1057	O	LEU	142	-23.764	-45.680	43.099	1.000	20.55
ATOM	1058	N	ALA	143	-23.363	-43.670	42.126	1.000	15.81
ATOM	1059	CA	ALA	143	-22.960	-42.941	43.322	1.000	13.69
ATOM	1060	CB	ALA	143	-21.461	-42.676	43.239	1.000	3.16
ATOM	1061	C	ALA	143	-23.762	-41.656	43.475	1.000	16.69

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ATOM	1062	O	ALA	143	-24.500	-41.280	42.552	1.000	10.61
ATOM	1063	N	PRO	144	-23.668	-40.968	44.609	1.000	19.19
ATOM	1064	CD	PRO	144	-22.997	-41.377	45.852	1.000	16.93
ATOM	1065	CA	PRO	144	-24.315	-39.659	44.745	1.000	19.29
ATOM	1066	CB	PRO	144	-23.730	-39.076	46.031	1.000	17.13
ATOM	1067	CG	PRO	144	-22.904	-40.130	46.664	1.000	12.97
ATOM	1068	C	PRO	144	-24.009	-38.723	43.578	1.000	17.14
ATOM	1069	O	PRO	144	-22.902	-38.626	43.048	1.000	12.89
ATOM	1070	N	MET	145	-25.049	-38.002	43.161	1.000	18.09
ATOM	1071	CA	MET	145	-24.925	-37.064	42.052	1.000	14.70
ATOM	1072	CB	MET	145	-25.912	-37.398	40.942	1.000	21.06
ATOM	1073	CG	MET	145	-25.711	-38.740	40.263	1.000	24.88
ATOM	1074	XD	MET	145	-27.259	-39.577	39.860	1.000	18.47
ATOM	1075	CE	MET	145	-27.956	-39.804	41.495	1.000	34.91
ATOM	1076	C	MET	145	-25.155	-35.645	42.559	1.000	11.49
ATOM	1077	O	MET	145	-26.205	-35.342	43.116	1.000	18.46
ATOM	1078	N	PRO	146	-24.182	-34.763	42.367	1.000	6.41
ATOM	1079	CD	PRO	146	-22.909	-34.993	41.683	1.000	8.62
ATOM	1080	CA	PRO	146	-24.325	-33.388	42.851	1.000	10.88
ATOM	1081	CB	PRO	146	-22.916	-32.814	42.759	1.000	10.59
ATOM	1082	CG	PRO	146	-22.064	-33.819	42.072	1.000	12.17
ATOM	1083	C	PRO	146	-25.292	-32.588	41.972	1.000	13.13
ATOM	1084	O	PRO	146	-25.999	-31.712	42.484	1.000	17.39
ATOM	1085	N	HIS	147	-25.311	-32.901	40.677	1.000	10.50
ATOM	1086	CA	HIS	147	-26.203	-32.215	39.758	1.000	9.69
ATOM	1087	CB	HIS	147	-25.865	-32.480	38.279	1.000	14.24
ATOM	1088	CG	HIS	147	-26.441	-31.373	37.431	1.000	6.69
ATOM	1089	CD2	HIS	147	-25.875	-30.297	36.850	1.000	5.99
ATOM	1090	ND1	HIS	147	-27.780	-31.296	37.134	1.000	11.40
ATOM	1091	CE1	HIS	147	-28.018	-30.226	36.391	1.000	11.68
ATOM	1092	NE2	HIS	147	-26.871	-29.600	36.201	1.000	12.68
ATOM	1093	C	HIS	147	-27.658	-32.596	40.013	1.000	5.47
ATOM	1094	O	HIS	147	-28.052	-33.761	39.960	1.000	11.15
ATOM	1095	N	PRO	148	-28.463	-31.575	40.291	1.000	12.88
ATOM	1096	CD	PRO	148	-28.098	-30.148	40.322	1.000	12.98
ATOM	1097	CA	PRO	148	-29.877	-31.806	40.602	1.000	13.30
ATOM	1098	CB	PRO	148	-30.440	-30.401	40.811	1.000	14.82
ATOM	1099	CG	PRO	148	-29.426	-29.455	40.267	1.000	16.64
ATOM	1100	C	PRO	148	-30.600	-32.508	39.456	1.000	15.39
ATOM	1101	O	PRO	148	-31.525	-33.290	39.689	1.000	15.71
ATOM	1102	N	TRP	149	-30.218	-32.263	38.201	1.000	21.29
ATOM	1103	CA	TRP	149	-30.909	-32.947	37.109	1.000	15.64
ATOM	1104	CB	TRP	149	-30.571	-32.328	35.750	1.000	17.31
ATOM	1105	CG	TRP	149	-31.296	-33.043	34.639	1.000	10.06
ATOM	1106	CD2	TRP	149	-32.715	-33.086	34.444	1.000	4.30
ATOM	1107	CE2	TRP	149	-32.952	-33.862	33.295	1.000	8.55
ATOM	1108	CE3	TRP	149	-33.805	-32.541	35.129	1.000	4.24

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ATOM   1109  CD1 TRP   149      -30.748 -33.774  33.629 1.000 11.09
ATOM   1110  NE1 TRP   149      -31.736 -34.272  32.813 1.000  5.61
ATOM   1111  CZ2 TRP   149      -34.240 -34.107  32.815 1.000 12.36
ATOM   1112  CZ3 TRP   149      -35.076 -32.785  34.654 1.000 13.41
ATOM   1113  CH2 TRP   149      -35.286 -33.563  33.505 1.000 14.13
ATOM   1114  C   TRP   149      -30.566 -34.432  37.101 1.000 12.85
ATOM   1115  O   TRP   149      -31.447 -35.290  37.033 1.000  7.92
ATOM   1116  N   PHE   150      -29.270 -34.728  37.186 1.000 11.11
ATOM   1117  CA  PHE   150      -28.841 -36.125  37.305 1.000 11.76
ATOM   1118  CB  PHE   150      -27.321 -36.192  37.483 1.000  8.65
ATOM   1119  CG  PHE   150      -26.581 -36.170  36.150 1.000 13.44
ATOM   1120  CD1 PHE   150      -25.315 -35.623  36.047 1.000 14.41
ATOM   1121  CD2 PHE   150      -27.167 -36.697  35.014 1.000 12.01
ATOM   1122  CE1 PHE   150      -24.650 -35.604  34.838 1.000 14.96
ATOM   1123  CE2 PHE   150      -26.511 -36.684  33.797 1.000 13.41
ATOM   1124  CZ  PHE   150      -25.246 -36.136  33.711 1.000 18.95
ATOM   1125  C   PHE   150      -29.555 -36.813  38.459 1.000 10.90
ATOM   1126  O   PHE   150      -30.059 -37.930  38.354 1.000  7.95
ATOM   1127  N   GLN   151      -29.606 -36.120  39.598 1.000 12.36
ATOM   1128  CA  GLN   151      -30.294 -36.665  40.759 1.000 19.45
ATOM   1129  CB  GLN   151      -30.306 -35.680  41.932 1.000 12.11
ATOM   1130  CG  GLN   151      -28.947 -35.446  42.561 1.000 16.34
ATOM   1131  CD  GLN   151      -29.048 -34.481  43.734 1.000 22.05
ATOM   1132  OE1 GLN   151      -29.693 -34.803  44.729 1.000 39.76
ATOM   1133  NE2 GLN   151      -28.423 -33.317  43.598 1.000 16.49
ATOM   1134  C   GLN   151      -31.745 -37.027  40.441 1.000 20.77
ATOM   1135  O   GLN   151      -32.232 -38.044  40.936 1.000 19.36
ATOM   1136  N   LEU   152      -32.397 -36.183  39.644 1.000 11.67
ATOM   1137  CA  LEU   152      -33.818 -36.360  39.365 1.000 13.95
ATOM   1138  CB  LEU   152      -34.438 -35.101  38.764 1.000 14.14
ATOM   1139  CG  LEU   152      -34.837 -33.957  39.688 1.000 12.09
ATOM   1140  CD1 LEU   152      -34.781 -32.631  38.935 1.000 11.66
ATOM   1141  CD2 LEU   152      -36.225 -34.162  40.274 1.000 12.14
ATOM   1142  C   LEU   152      -34.053 -37.544  38.428 1.000 13.07
ATOM   1143  O   LEU   152      -34.913 -38.372  38.729 1.000 13.96
ATOM   1144  N   ILE   153      -33.310 -37.613  37.326 1.000 13.21
ATOM   1145  CA  ILE   153      -33.519 -38.661  36.334 1.000 12.12
ATOM   1146  CB  ILE   153      -32.814 -38.377  34.991 1.000  9.74
ATOM   1147  CG2 ILE   153      -33.360 -37.106  34.355 1.000  0.00
ATOM   1148  CG1 ILE   153      -31.284 -38.333  35.061 1.000  8.16
ATOM   1149  CD1 ILE   153      -30.635 -38.332  33.684 1.000  0.00
ATOM   1150  C   ILE   153      -33.054 -40.024  36.836 1.000  9.56
ATOM   1151  O   ILE   153      -33.540 -41.043  36.342 1.000  4.79
ATOM   1152  N   PHE   154      -32.138 -40.069  37.797 1.000 12.41
ATOM   1153  CA  PHE   154      -31.645 -41.349  38.301 1.000  8.75
ATOM   1154  CB  PHE   154      -30.113 -41.372  38.348 1.000  8.88
ATOM   1155  CG  PHE   154      -29.456 -41.758  37.031 1.000  8.38
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ATOM   1156  CD1 PHE   154      -28.597 -40.887  36.384 1.000  9.10
ATOM   1157  CD2 PHE   154      -29.703 -42.990  36.458 1.000  0.00
ATOM   1158  CE1 PHE   154      -28.000 -41.232  35.188 1.000  9.85
ATOM   1159  CE2 PHE   154      -29.119 -43.344  35.260 1.000  5.02
ATOM   1160  CZ  PHE   154      -28.258 -42.468  34.624 1.000  8.39
ATOM   1161  C   PHE   154      -32.199 -41.648  39.690 1.000 11.55
ATOM   1162  O   PHE   154      -31.683 -42.515  40.400 1.000 10.77
ATOM   1163  N   GLU   155      -33.246 -40.936  40.093 1.000 15.11
ATOM   1164  CA  GLU   155      -33.898 -41.221  41.367 1.000 19.95
ATOM   1165  CB  GLU   155      -35.134 -40.343  41.542 1.000 26.08
ATOM   1166  CG  GLU   155      -35.558 -40.107  42.980 1.000 33.00
ATOM   1167  CD  GLU   155      -36.339 -41.267  43.568 1.000 44.51
ATOM   1168  OE1 GLU   155      -37.432 -41.585  43.051 1.000 49.47
ATOM   1169  OE2 GLU   155      -35.862 -41.867  44.558 1.000 61.39
ATOM   1170  C   GLU   155      -34.270 -42.702  41.449 1.000 18.82
ATOM   1171  O   GLU   155      -34.978 -43.212  40.582 1.000 14.49
ATOM   1172  N   GLY   156      -33.779 -43.376  42.481 1.000 12.58
ATOM   1173  CA  GLY   156      -33.993 -44.787  42.696 1.000  6.50
ATOM   1174  C   GLY   156      -33.061 -45.684  41.914 1.000 12.22
ATOM   1175  O   GLY   156      -33.205 -46.914  41.914 1.000 27.90
ATOM   1176  N   GLY   157      -32.082 -45.107  41.224 1.000  9.19
ATOM   1177  CA  GLY   157      -31.216 -45.877  40.358 1.000  8.21
ATOM   1178  C   GLY   157      -30.007 -46.514  40.991 1.000  8.61
ATOM   1179  O   GLY   157      -29.563 -47.579  40.549 1.000 17.22
ATOM   1180  N   GLU   158      -29.442 -45.887  42.018 1.000  7.58
ATOM   1181  CA  GLU   158      -28.299 -46.453  42.721 1.000  7.50
ATOM   1182  CB  GLU   158      -27.807 -45.505  43.814 1.000  9.84
ATOM   1183  CG  GLU   158      -26.756 -46.097  44.739 1.000 11.00
ATOM   1184  CD  GLU   158      -26.031 -45.053  45.564 1.000 24.40
ATOM   1185  OE1 GLU   158      -26.158 -43.845  45.267 1.000 33.57
ATOM   1186  OE2 GLU   158      -25.325 -45.439  46.523 1.000 39.11
ATOM   1187  C   GLU   158      -28.696 -47.807  43.302 1.000 13.34
ATOM   1188  O   GLU   158      -27.956 -48.787  43.225 1.000 29.78
ATOM   1189  N   GLN   159      -29.895 -47.840  43.875 1.000 10.17
ATOM   1190  CA  GLN   159      -30.481 -49.058  44.406 1.000 15.50
ATOM   1191  CB  GLN   159      -31.856 -48.764  45.017 1.000 19.57
ATOM   1192  CG  GLN   159      -32.548 -49.952  45.647 1.000 24.93
ATOM   1193  CD  GLN   159      -31.737 -50.676  46.704 1.000 30.24
ATOM   1194  OE1 GLN   159      -31.940 -50.499  47.909 1.000 40.80
ATOM   1195  NE2 GLN   159      -30.800 -51.510  46.265 1.000 20.75
ATOM   1196  C   GLN   159      -30.605 -50.132  43.336 1.000 17.89
ATOM   1197  O   GLN   159      -30.218 -51.285  43.544 1.000 21.71
ATOM   1198  N   LYS   160      -31.154 -49.791  42.168 1.000 15.99
ATOM   1199  CA  LYS   160      -31.361 -50.855  41.176 1.000  6.75
ATOM   1200  CB  LYS   160      -32.314 -50.369  40.090 1.000 10.24
ATOM   1201  CG  LYS   160      -33.666 -49.907  40.607 1.000  6.13
ATOM   1202  CD  LYS   160      -34.386 -49.041  39.581 1.000 11.21
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ATOM	1203	CE	LYS	160	-35.897	-49.190	39.702	1.000	9.55
ATOM	1204	NZ	LYS	160	-36.616	-48.235	38.811	1.000	20.37
ATOM	1205	C	LYS	160	-30.029	-51.305	40.591	1.000	14.32
ATOM	1206	O	LYS	160	-29.842	-52.475	40.257	1.000	14.42
ATOM	1207	N	THR	161	-29.082	-50.375	40.465	1.000	10.29
ATOM	1208	CA	THR	161	-27.771	-50.734	39.933	1.000	13.43
ATOM	1209	CB	THR	161	-26.878	-49.508	39.672	1.000	10.03
ATOM	1210	OG1	THR	161	-27.070	-48.557	40.730	1.000	30.01
ATOM	1211	CG2	THR	161	-27.263	-48.788	38.389	1.000	13.57
ATOM	1212	C	THR	161	-27.057	-51.683	40.896	1.000	12.06
ATOM	1213	O	THR	161	-26.160	-52.415	40.481	1.000	6.51
ATOM	1214	N	THR	162	-27.457	-51.664	42.165	1.000	8.39
ATOM	1215	CA	THR	162	-26.894	-52.551	43.177	1.000	9.75
ATOM	1216	CB	THR	162	-27.286	-52.130	44.604	1.000	12.96
ATOM	1217	OG1	THR	162	-26.705	-50.863	44.941	1.000	11.98
ATOM	1218	CG2	THR	162	-26.735	-53.132	45.605	1.000	20.35
ATOM	1219	C	THR	162	-27.349	-53.991	42.956	1.000	10.87
ATOM	1220	O	THR	162	-26.764	-54.942	43.471	1.000	12.87
ATOM	1221	N	GLU	163	-28.410	-54.170	42.174	1.000	16.58
ATOM	1222	CA	GLU	163	-28.949	-55.496	41.905	1.000	20.69
ATOM	1223	CB	GLU	163	-30.486	-55.450	41.861	1.000	21.36
ATOM	1224	CG	GLU	163	-31.136	-54.918	43.122	1.000	19.81
ATOM	1225	CD	GLU	163	-30.918	-55.799	44.332	1.000	20.57
ATOM	1226	OE1	GLU	163	-30.336	-56.894	44.181	1.000	13.38
ATOM	1227	OE2	GLU	163	-31.340	-55.394	45.441	1.000	37.36
ATOM	1228	C	GLU	163	-28.455	-56.101	40.596	1.000	12.31
ATOM	1229	O	GLU	163	-28.614	-57.306	40.384	1.000	8.17
ATOM	1230	N	LEU	164	-27.880	-55.296	39.710	1.000	14.12
ATOM	1231	CA	LEU	164	-27.561	-55.746	38.356	1.000	8.92
ATOM	1232	CB	LEU	164	-26.960	-54.602	37.541	1.000	5.54
ATOM	1233	CG	LEU	164	-27.903	-53.857	36.593	1.000	10.39
ATOM	1234	CD1	LEU	164	-29.295	-53.740	37.197	1.000	23.43
ATOM	1235	CD2	LEU	164	-27.352	-52.485	36.240	1.000	2.48
ATOM	1236	C	LEU	164	-26.621	-56.943	38.361	1.000	6.54
ATOM	1237	O	LEU	164	-26.847	-57.925	37.653	1.000	4.26
ATOM	1238	N	ALA	165	-25.562	-56.865	39.159	1.000	7.24
ATOM	1239	CA	ALA	165	-24.609	-57.965	39.239	1.000	11.41
ATOM	1240	CB	ALA	165	-23.542	-57.659	40.276	1.000	11.40
ATOM	1241	C	ALA	165	-25.312	-59.284	39.551	1.000	16.26
ATOM	1242	O	ALA	165	-24.980	-60.302	38.947	1.000	18.13
ATOM	1243	N	ARG	166	-26.266	-59.245	40.469	1.000	20.04
ATOM	1244	CA	ARG	166	-27.014	-60.397	40.947	1.000	10.10
ATOM	1245	CB	ARG	166	-27.875	-59.992	42.145	1.000	15.40
ATOM	1246	CG	ARG	166	-28.600	-61.127	42.843	1.000	15.67
ATOM	1247	CD	ARG	166	-29.286	-60.640	44.115	1.000	20.34
ATOM	1248	NE	ARG	166	-30.097	-59.453	43.851	1.000	31.99
ATOM	1249	CZ	ARG	166	-31.261	-59.505	43.202	1.000	37.46

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ATOM    1250  NH1 ARG    166      -31.718 -60.673  42.770 1.000 41.26
ATOM    1251  NH2 ARG    166      -31.974 -58.410  42.979 1.000 44.85
ATOM    1252  C   ARG    166      -27.899 -60.991  39.862 1.000 10.33
ATOM    1253  O   ARG    166      -27.862 -62.186  39.569 1.000 11.28
ATOM    1254  N   VAL    167      -28.724 -60.143  39.253 1.000 10.14
ATOM    1255  CA  VAL    167      -29.647 -60.637  38.231 1.000  8.08
ATOM    1256  CB  VAL    167      -30.800 -59.642  38.007 1.000 12.63
ATOM    1257  CG1 VAL    167      -31.873 -60.262  37.129 1.000 23.15
ATOM    1258  CG2 VAL    167      -31.423 -59.212  39.331 1.000 16.49
ATOM    1259  C   VAL    167      -28.941 -60.943  36.916 1.000  8.93
ATOM    1260  O   VAL    167      -29.342 -61.889  36.230 1.000 11.00
ATOM    1261  N   TYR    168      -27.906 -60.209  36.507 1.000  6.53
ATOM    1262  CA  TYR    168      -27.225 -60.549  35.262 1.000  5.82
ATOM    1263  CB  TYR    168      -26.220 -59.494  34.815 1.000 12.35
ATOM    1264  CG  TYR    168      -26.746 -58.249  34.148 1.000 10.53
ATOM    1265  CD1 TYR    168      -25.898 -57.415  33.429 1.000  4.25
ATOM    1266  CE1 TYR    168      -26.377 -56.273  32.816 1.000  3.59
ATOM    1267  CD2 TYR    168      -28.085 -57.889  34.230 1.000  9.22
ATOM    1268  CE2 TYR    168      -28.565 -56.750  33.624 1.000 11.67
ATOM    1269  CZ  TYR    168      -27.708 -55.940  32.912 1.000  8.76
ATOM    1270  OH  TYR    168      -28.194 -54.801  32.308 1.000 13.56
ATOM    1271  C   TYR    168      -26.466 -61.863  35.444 1.000  9.45
ATOM    1272  O   TYR    168      -26.398 -62.696  34.544 1.000  5.20
ATOM    1273  N   SER    169      -25.896 -61.972  36.648 1.000  5.94
ATOM    1274  CA  SER    169      -25.145 -63.174  36.999 1.000 11.65
ATOM    1275  CB  SER    169      -24.663 -63.109  38.445 1.000 12.52
ATOM    1276  OG  SER    169      -23.611 -64.024  38.688 1.000 13.86
ATOM    1277  C   SER    169      -26.034 -64.389  36.740 1.000 14.93
ATOM    1278  O   SER    169      -25.709 -65.240  35.912 1.000 25.35
ATOM    1279  N   ALA    170      -27.161 -64.434  37.448 1.000  9.54
ATOM    1280  CA  ALA    170      -28.154 -65.483  37.259 1.000  7.33
ATOM    1281  CB  ALA    170      -29.397 -65.155  38.069 1.000  3.12
ATOM    1282  C   ALA    170      -28.495 -65.659  35.785 1.000 12.27
ATOM    1283  O   ALA    170      -28.526 -66.772  35.262 1.000 20.56
ATOM    1284  N   LEU    171      -28.753 -64.558  35.081 1.000 15.11
ATOM    1285  CA  LEU    171      -29.115 -64.661  33.665 1.000 17.04
ATOM    1286  CB  LEU    171      -29.329 -63.272  33.076 1.000 13.64
ATOM    1287  CG  LEU    171      -29.846 -63.164  31.645 1.000 21.08
ATOM    1288  CD1 LEU    171      -28.692 -63.043  30.658 1.000 45.18
ATOM    1289  CD2 LEU    171      -30.734 -64.340  31.270 1.000 17.34
ATOM    1290  C   LEU    171      -28.052 -65.404  32.868 1.000 18.57
ATOM    1291  O   LEU    171      -28.328 -66.409  32.219 1.000 17.64
ATOM    1292  N   ALA    172      -26.825 -64.890  32.920 1.000 22.46
ATOM    1293  CA  ALA    172      -25.735 -65.489  32.157 1.000 17.47
ATOM    1294  CB  ALA    172      -24.454 -64.699  32.377 1.000 10.29
ATOM    1295  C   ALA    172      -25.549 -66.953  32.536 1.000 13.15
ATOM    1296  O   ALA    172      -25.192 -67.797  31.713 1.000 17.25
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ATOM   1297  N   SER   173      -25.802 -67.242  33.809 1.000 11.55
ATOM   1298  CA  SER   173      -25.653 -68.595  34.337 1.000 15.80
ATOM   1299  CB  SER   173      -25.837 -68.578  35.856 1.000 15.14
ATOM   1300  OG  SER   173      -26.298 -69.837  36.293 1.000 15.66
ATOM   1301  C   SER   173      -26.640 -69.565  33.691 1.000 10.39
ATOM   1302  O   SER   173      -26.263 -70.667  33.284 1.000  5.06
ATOM   1303  N   PHE   174      -27.882 -69.119  33.601 1.000  6.57
ATOM   1304  CA  PHE   174      -28.970 -69.778  32.908 1.000  4.04
ATOM   1305  CB  PHE   174      -30.288 -69.024  33.114 1.000  4.43
ATOM   1306  CG  PHE   174      -31.524 -69.765  32.626 1.000  3.57
ATOM   1307  CD1 PHE   174      -32.219 -70.606  33.475 1.000  0.40
ATOM   1308  CD2 PHE   174      -31.988 -69.615  31.331 1.000 11.71
ATOM   1309  CE1 PHE   174      -33.343 -71.281  33.051 1.000  1.63
ATOM   1310  CE2 PHE   174      -33.114 -70.285  30.886 1.000 10.57
ATOM   1311  CZ  PHE   174      -33.795 -71.119  31.756 1.000 10.59
ATOM   1312  C   PHE   174      -28.701 -69.872  31.408 1.000  8.80
ATOM   1313  O   PHE   174      -28.846 -70.949  30.834 1.000  0.14
ATOM   1314  N   MET   175      -28.328 -68.751  30.793 1.000  7.91
ATOM   1315  CA  MET   175      -28.058 -68.739  29.356 1.000  5.97
ATOM   1316  CB  MET   175      -28.103 -67.321  28.780 1.000  0.00
ATOM   1317  CG  MET   175      -29.492 -66.712  28.751 1.000  7.42
ATOM   1318 XD   MET   175      -29.573 -65.056  28.023 1.000 16.37
ATOM   1319  CE  MET   175      -30.064 -65.488  26.348 1.000 21.02
ATOM   1320  C   MET   175      -26.715 -69.399  29.045 1.000  6.31
ATOM   1321  O   MET   175      -26.332 -69.479  27.880 1.000  8.17
ATOM   1322  N   LYS   176      -26.020 -69.872  30.070 1.000  8.77
ATOM   1323  CA  LYS   176      -24.762 -70.598  29.939 1.000 10.68
ATOM   1324  CB  LYS   176      -24.970 -71.945  29.239 1.000 10.45
ATOM   1325  CG  LYS   176      -25.907 -72.900  29.971 1.000  3.74
ATOM   1326  CD  LYS   176      -25.133 -73.755  30.964 1.000  5.05
ATOM   1327  CE  LYS   176      -26.084 -74.568  31.833 1.000  6.09
ATOM   1328  NZ  LYS   176      -26.739 -73.721  32.861 1.000 24.38
ATOM   1329  C   LYS   176      -23.733 -69.760  29.190 1.000 12.34
ATOM   1330  O   LYS   176      -23.084 -70.178  28.231 1.000 24.85
ATOM   1331  N   VAL   177      -23.601 -68.520  29.648 1.000 12.09
ATOM   1332  CA  VAL   177      -22.709 -67.581  28.953 1.000 12.10
ATOM   1333  CB  VAL   177      -23.569 -66.629  28.106 1.000  9.74
ATOM   1334  CG1 VAL   177      -23.831 -65.319  28.835 1.000 18.59
ATOM   1335  CG2 VAL   177      -22.921 -66.372  26.753 1.000 20.30
ATOM   1336  C   VAL   177      -21.848 -66.876  29.982 1.000 13.62
ATOM   1337  O   VAL   177      -22.292 -66.730  31.126 1.000 20.25
ATOM   1338  N   PRO   178      -20.635 -66.454  29.637 1.000 10.56
ATOM   1339  CD  PRO   178      -20.019 -66.530  28.312 1.000  2.11
ATOM   1340  CA  PRO   178      -19.760 -65.842  30.642 1.000 10.32
ATOM   1341  CB  PRO   178      -18.433 -65.656  29.913 1.000  6.70
ATOM   1342  CG  PRO   178      -18.623 -66.026  28.499 1.000  0.81
ATOM   1343  C   PRO   178      -20.281 -64.483  31.119 1.000 20.65
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ATOM	1344	O	PRO	178	-20.796	-63.674	30.351	1.000	22.70
ATOM	1345	N	PHE	179	-20.124	-64.253	32.412	1.000	22.55
ATOM	1346	CA	PHE	179	-20.474	-63.025	33.107	1.000	19.13
ATOM	1347	CB	PHE	179	-21.518	-63.283	34.194	1.000	8.91
ATOM	1348	CG	PHE	179	-21.661	-62.215	35.268	1.000	8.12
ATOM	1349	CD1	PHE	179	-22.433	-61.087	35.044	1.000	10.36
ATOM	1350	CD2	PHE	179	-21.031	-62.337	36.499	1.000	2.04
ATOM	1351	CE1	PHE	179	-22.590	-60.103	36.004	1.000	2.43
ATOM	1352	CE2	PHE	179	-21.183	-61.367	37.470	1.000	0.76
ATOM	1353	CZ	PHE	179	-21.963	-60.248	37.228	1.000	2.96
ATOM	1354	C	PHE	179	-19.231	-62.400	33.736	1.000	13.74
ATOM	1355	O	PHE	179	-18.309	-63.110	34.128	1.000	15.60
ATOM	1356	N	PHE	180	-19.214	-61.080	33.838	1.000	14.28
ATOM	1357	CA	PHE	180	-18.178	-60.371	34.573	1.000	13.03
ATOM	1358	CB	PHE	180	-17.004	-59.952	33.686	1.000	17.94
ATOM	1359	CG	PHE	180	-15.933	-59.164	34.433	1.000	21.76
ATOM	1360	CD1	PHE	180	-14.960	-59.807	35.176	1.000	21.38
ATOM	1361	CD2	PHE	180	-15.904	-57.780	34.391	1.000	19.62
ATOM	1362	CE1	PHE	180	-13.979	-59.108	35.859	1.000	15.07
ATOM	1363	CE2	PHE	180	-14.941	-57.064	35.075	1.000	21.73
ATOM	1364	CZ	PHE	180	-13.979	-57.727	35.816	1.000	21.65
ATOM	1365	C	PHE	180	-18.822	-59.164	35.256	1.000	12.16
ATOM	1366	O	PHE	180	-19.594	-58.423	34.648	1.000	11.01
ATOM	1367	N	ASP	181	-18.504	-58.988	36.536	1.000	7.72
ATOM	1368	CA	ASP	181	-19.062	-57.864	37.286	1.000	10.61
ATOM	1369	CB	ASP	181	-19.521	-58.346	38.659	1.000	5.77
ATOM	1370	CG	ASP	181	-19.986	-57.225	39.559	1.000	4.11
ATOM	1371	OD1	ASP	181	-20.116	-56.076	39.092	1.000	8.61
ATOM	1372	OD2	ASP	181	-20.217	-57.508	40.750	1.000	11.49
ATOM	1373	C	ASP	181	-18.037	-56.743	37.378	1.000	15.44
ATOM	1374	O	ASP	181	-17.023	-56.872	38.060	1.000	16.84
ATOM	1375	N	ALA	182	-18.293	-55.639	36.672	1.000	18.65
ATOM	1376	CA	ALA	182	-17.359	-54.517	36.678	1.000	18.00
ATOM	1377	CB	ALA	182	-17.778	-53.459	35.668	1.000	7.66
ATOM	1378	C	ALA	182	-17.240	-53.911	38.075	1.000	18.92
ATOM	1379	O	ALA	182	-16.198	-53.340	38.400	1.000	8.61
ATOM	1380	N	GLY	183	-18.296	-54.044	38.872	1.000	15.67
ATOM	1381	CA	GLY	183	-18.374	-53.516	40.219	1.000	13.53
ATOM	1382	C	GLY	183	-17.444	-54.230	41.176	1.000	14.96
ATOM	1383	O	GLY	183	-17.268	-53.846	42.330	1.000	25.31
ATOM	1384	N	SER	184	-16.830	-55.306	40.696	1.000	16.38
ATOM	1385	CA	SER	184	-15.940	-56.105	41.525	1.000	12.32
ATOM	1386	CB	SER	184	-16.009	-57.574	41.116	1.000	14.55
ATOM	1387	OG	SER	184	-15.237	-57.867	39.967	1.000	12.36
ATOM	1388	C	SER	184	-14.516	-55.572	41.439	1.000	13.33
ATOM	1389	O	SER	184	-13.644	-55.986	42.204	1.000	12.05
ATOM	1390	N	VAL	185	-14.276	-54.640	40.515	1.000	9.89

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ATOM	1391	CA	VAL	185	-12.902	-54.156	40.358	1.000	14.54
ATOM	1392	CB	VAL	185	-12.320	-54.649	39.021	1.000	16.34
ATOM	1393	CG1	VAL	185	-12.034	-56.141	39.100	1.000	13.09
ATOM	1394	CG2	VAL	185	-13.274	-54.346	37.877	1.000	20.34
ATOM	1395	C	VAL	185	-12.802	-52.642	40.445	1.000	20.13
ATOM	1396	O	VAL	185	-11.718	-52.101	40.682	1.000	11.67
ATOM	1397	N	ILE	186	-13.912	-51.929	40.260	1.000	19.83
ATOM	1398	CA	ILE	186	-13.905	-50.479	40.381	1.000	13.97
ATOM	1399	CB	ILE	186	-13.716	-49.752	39.031	1.000	8.30
ATOM	1400	CG2	ILE	186	-12.362	-50.070	38.428	1.000	12.39
ATOM	1401	CG1	ILE	186	-14.830	-50.005	38.014	1.000	10.45
ATOM	1402	CD1	ILE	186	-14.956	-48.929	36.957	1.000	3.60
ATOM	1403	C	ILE	186	-15.209	-49.957	40.979	1.000	13.38
ATOM	1404	O	ILE	186	-16.256	-50.583	40.857	1.000	12.90
ATOM	1405	N	SER	187	-15.120	-48.788	41.596	1.000	11.99
ATOM	1406	CA	SER	187	-16.287	-48.046	42.052	1.000	9.16
ATOM	1407	CB	SER	187	-16.110	-47.594	43.498	1.000	10.88
ATOM	1408	OG	SER	187	-14.889	-46.879	43.658	1.000	16.58
ATOM	1409	C	SER	187	-16.517	-46.839	41.145	1.000	11.87
ATOM	1410	O	SER	187	-15.567	-46.304	40.563	1.000	16.73
ATOM	1411	N	THR	188	-17.767	-46.410	41.015	1.000	15.17
ATOM	1412	CA	THR	188	-18.077	-45.244	40.189	1.000	13.51
ATOM	1413	CB	THR	188	-19.571	-45.151	39.848	1.000	12.88
ATOM	1414	OG1	THR	188	-19.969	-46.308	39.101	1.000	16.33
ATOM	1415	CG2	THR	188	-19.843	-43.943	38.961	1.000	8.08
ATOM	1416	C	THR	188	-17.639	-43.978	40.916	1.000	14.09
ATOM	1417	O	THR	188	-18.293	-43.535	41.860	1.000	10.72
ATOM	1418	N	ASP	189	-16.518	-43.414	40.474	1.000	15.51
ATOM	1419	CA	ASP	189	-15.911	-42.313	41.210	1.000	11.58
ATOM	1420	CB	ASP	189	-14.407	-42.594	41.362	1.000	12.86
ATOM	1421	CG	ASP	189	-14.158	-43.791	42.261	1.000	4.55
ATOM	1422	OD1	ASP	189	-14.915	-43.960	43.239	1.000	13.27
ATOM	1423	OD2	ASP	189	-13.208	-44.549	41.989	1.000	6.91
ATOM	1424	C	ASP	189	-16.120	-40.949	40.567	1.000	15.34
ATOM	1425	O	ASP	189	-15.910	-39.948	41.263	1.000	18.48
ATOM	1426	N	GLY	190	-16.510	-40.918	39.303	1.000	19.39
ATOM	1427	CA	GLY	190	-16.710	-39.718	38.515	1.000	15.08
ATOM	1428	C	GLY	190	-17.385	-38.613	39.303	1.000	18.57
ATOM	1429	O	GLY	190	-18.263	-38.908	40.119	1.000	20.64
ATOM	1430	N	VAL	191	-16.952	-37.381	39.057	1.000	13.86
ATOM	1431	CA	VAL	191	-17.428	-36.226	39.806	1.000	10.59
ATOM	1432	CB	VAL	191	-16.825	-34.905	39.286	1.000	17.05
ATOM	1433	CG1	VAL	191	-15.324	-34.875	39.559	1.000	30.84
ATOM	1434	CG2	VAL	191	-17.092	-34.701	37.803	1.000	8.10
ATOM	1435	C	VAL	191	-18.950	-36.129	39.774	1.000	10.47
ATOM	1436	O	VAL	191	-19.542	-35.686	40.761	1.000	13.60
ATOM	1437	N	ASP	192	-19.571	-36.534	38.668	1.000	1.46

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ATOM	1438	CA	ASP	192	-21.018	-36.447	38.540	1.000	0.70
ATOM	1439	CB	ASP	192	-21.387	-36.356	37.056	1.000	2.10
ATOM	1440	CG	ASP	192	-20.918	-37.566	36.268	1.000	9.82
ATOM	1441	OD1	ASP	192	-20.296	-38.478	36.857	1.000	8.20
ATOM	1442	OD2	ASP	192	-21.182	-37.597	35.047	1.000	6.78
ATOM	1443	C	ASP	192	-21.754	-37.622	39.173	1.000	7.73
ATOM	1444	O	ASP	192	-22.988	-37.674	39.136	1.000	7.10
ATOM	1445	N	GLY	193	-21.027	-38.572	39.753	1.000	15.10
ATOM	1446	CA	GLY	193	-21.631	-39.747	40.351	1.000	17.83
ATOM	1447	C	GLY	193	-22.153	-40.758	39.352	1.000	18.93
ATOM	1448	O	GLY	193	-22.820	-41.732	39.718	1.000	10.12
ATOM	1449	N	ILE	194	-21.867	-40.565	38.062	1.000	11.77
ATOM	1450	CA	ILE	194	-22.330	-41.546	37.081	1.000	7.87
ATOM	1451	CB	ILE	194	-23.401	-40.945	36.154	1.000	9.95
ATOM	1452	CG2	ILE	194	-23.790	-41.927	35.063	1.000	0.00
ATOM	1453	CG1	ILE	194	-24.643	-40.441	36.896	1.000	9.90
ATOM	1454	CD1	ILE	194	-25.248	-39.237	36.206	1.000	8.85
ATOM	1455	C	ILE	194	-21.191	-42.068	36.225	1.000	2.97
ATOM	1456	O	ILE	194	-21.086	-43.251	35.924	1.000	6.72
ATOM	1457	N	HIS	195	-20.277	-41.195	35.792	1.000	6.33
ATOM	1458	CA	HIS	195	-19.256	-41.719	34.884	1.000	10.76
ATOM	1459	CB	HIS	195	-19.089	-40.790	33.673	1.000	11.36
ATOM	1460	CG	HIS	195	-20.402	-40.647	32.958	1.000	11.50
ATOM	1461	CD2	HIS	195	-20.981	-41.395	31.989	1.000	5.43
ATOM	1462	ND1	HIS	195	-21.283	-39.633	33.253	1.000	7.30
ATOM	1463	CE1	HIS	195	-22.351	-39.753	32.485	1.000	9.11
ATOM	1464	NE2	HIS	195	-22.192	-40.814	31.711	1.000	8.18
ATOM	1465	C	HIS	195	-17.918	-41.941	35.577	1.000	8.63
ATOM	1466	O	HIS	195	-17.762	-41.602	36.743	1.000	13.71
ATOM	1467	N	PHE	196	-17.010	-42.529	34.812	1.000	6.37
ATOM	1468	CA	PHE	196	-15.725	-43.017	35.249	1.000	9.06
ATOM	1469	CB	PHE	196	-15.233	-44.136	34.320	1.000	5.38
ATOM	1470	CG	PHE	196	-16.048	-45.412	34.451	1.000	10.20
ATOM	1471	CD1	PHE	196	-15.822	-46.481	33.602	1.000	8.01
ATOM	1472	CD2	PHE	196	-17.027	-45.509	35.427	1.000	6.21
ATOM	1473	CE1	PHE	196	-16.571	-47.637	33.722	1.000	11.17
ATOM	1474	CE2	PHE	196	-17.779	-46.662	35.546	1.000	14.06
ATOM	1475	CZ	PHE	196	-17.549	-47.727	34.694	1.000	13.03
ATOM	1476	C	PHE	196	-14.663	-41.925	35.273	1.000	12.92
ATOM	1477	O	PHE	196	-14.757	-40.983	34.494	1.000	15.16
ATOM	1478	N	THR	197	-13.694	-42.112	36.158	1.000	13.17
ATOM	1479	CA	THR	197	-12.477	-41.318	36.183	1.000	17.95
ATOM	1480	CB	THR	197	-11.886	-41.168	37.593	1.000	20.94
ATOM	1481	OG1	THR	197	-11.650	-42.458	38.173	1.000	20.14
ATOM	1482	CG2	THR	197	-12.882	-40.454	38.499	1.000	31.55
ATOM	1483	C	THR	197	-11.443	-41.978	35.269	1.000	10.26
ATOM	1484	O	THR	197	-11.713	-43.037	34.705	1.000	14.53

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ATOM    1485   N   GLU    198       -10.283 -41.362  35.133 1.000  9.05
ATOM    1486   CA  GLU    198        -9.192 -41.943  34.362 1.000 12.89
ATOM    1487   CB  GLU    198        -8.023 -40.960  34.314 1.000 20.40
ATOM    1488   CG  GLU    198        -6.903 -41.349  33.362 1.000 32.30
ATOM    1489   CD  GLU    198        -5.764 -40.346  33.328 1.000 35.77
ATOM    1490   OE1 GLU    198        -5.127 -40.141  34.385 1.000 42.59
ATOM    1491   OE2 GLU    198        -5.498 -39.761  32.256 1.000 25.40
ATOM    1492   C   GLU    198        -8.779 -43.279  34.970 1.000 16.23
ATOM    1493   O   GLU    198        -8.636 -44.296  34.292 1.000 14.85
ATOM    1494   N   ALA    199        -8.596 -43.284  36.291 1.000 11.36
ATOM    1495   CA  ALA    199        -8.233 -44.489  37.022 1.000  5.99
ATOM    1496   CB  ALA    199        -8.047 -44.154  38.499 1.000  2.34
ATOM    1497   C   ALA    199        -9.273 -45.594  36.873 1.000  7.89
ATOM    1498   O   ALA    199        -8.922 -46.767  36.748 1.000 16.70
ATOM    1499   N   ASN    200       -10.548 -45.210  36.897 1.000 13.48
ATOM    1500   CA  ASN    200       -11.644 -46.155  36.715 1.000 11.59
ATOM    1501   CB  ASN    200       -13.007 -45.474  36.805 1.000  4.12
ATOM    1502   CG  ASN    200       -13.492 -45.192  38.209 1.000 11.67
ATOM    1503   OD1 ASN    200       -13.045 -45.767  39.200 1.000  6.19
ATOM    1504   ND2 ASN    200       -14.455 -44.276  38.330 1.000 13.74
ATOM    1505   C   ASN    200       -11.505 -46.869  35.366 1.000  8.88
ATOM    1506   O   ASN    200       -11.667 -48.084  35.305 1.000  9.08
ATOM    1507   N   ASN    201       -11.208 -46.111  34.315 1.000 14.48
ATOM    1508   CA  ASN    201       -11.074 -46.639  32.963 1.000 14.27
ATOM    1509   CB  ASN    201       -10.903 -45.495  31.960 1.000 16.17
ATOM    1510   CG  ASN    201       -12.221 -44.853  31.570 1.000 14.25
ATOM    1511   OD1 ASN    201       -13.050 -45.436  30.871 1.000 13.77
ATOM    1512   ND2 ASN    201       -12.441 -43.624  32.021 1.000 16.01
ATOM    1513   C   ASN    201        -9.908 -47.620  32.870 1.000 12.95
ATOM    1514   O   ASN    201       -10.050 -48.720  32.334 1.000 11.02
ATOM    1515   N   ARG    202        -8.775 -47.207  33.412 1.000 15.80
ATOM    1516   CA  ARG    202        -7.571 -48.020  33.532 1.000 14.85
ATOM    1517   CB  ARG    202        -6.491 -47.250  34.294 1.000 17.85
ATOM    1518   CG  ARG    202        -5.109 -47.874  34.325 1.000 17.66
ATOM    1519   CD  ARG    202        -4.141 -47.026  35.143 1.000 19.69
ATOM    1520   NE  ARG    202        -3.646 -45.881  34.388 1.000 30.64
ATOM    1521   CZ  ARG    202        -2.410 -45.407  34.412 1.000 36.54
ATOM    1522   NH1 ARG    202        -1.470 -45.972  35.164 1.000 35.38
ATOM    1523   NH2 ARG    202        -2.093 -44.353  33.669 1.000 23.31
ATOM    1524   C   ARG    202        -7.862 -49.344  34.229 1.000  6.52
ATOM    1525   O   ARG    202        -7.636 -50.401  33.644 1.000  9.98
ATOM    1526   N   ASP    203        -8.365 -49.285  35.464 1.000  3.83
ATOM    1527   CA  ASP    203        -8.597 -50.500  36.237 1.000 12.72
ATOM    1528   CB  ASP    203        -9.148 -50.181  37.631 1.000  9.96
ATOM    1529   CG  ASP    203        -8.170 -49.370  38.458 1.000 16.04
ATOM    1530   OD1 ASP    203        -6.980 -49.324  38.086 1.000 18.66
ATOM    1531   OD2 ASP    203        -8.584 -48.772  39.474 1.000 22.09
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ATOM	1532	C	ASP	203	-9.548	-51.455	35.524	1.000	18.07
ATOM	1533	O	ASP	203	-9.383	-52.674	35.579	1.000	12.38
ATOM	1534	N	LEU	204	-10.550	-50.890	34.859	1.000	23.73
ATOM	1535	CA	LEU	204	-11.541	-51.706	34.169	1.000	21.34
ATOM	1536	CB	LEU	204	-12.745	-50.872	33.727	1.000	26.39
ATOM	1537	CG	LEU	204	-14.123	-51.510	33.908	1.000	26.92
ATOM	1538	CD1	LEU	204	-15.079	-51.066	32.809	1.000	10.26
ATOM	1539	CD2	LEU	204	-14.019	-53.027	33.942	1.000	35.07
ATOM	1540	C	LEU	204	-10.938	-52.392	32.948	1.000	10.84
ATOM	1541	O	LEU	204	-11.212	-53.567	32.707	1.000	16.23
ATOM	1542	N	GLY	205	-10.143	-51.649	32.189	1.000	8.26
ATOM	1543	CA	GLY	205	-9.534	-52.173	30.984	1.000	6.27
ATOM	1544	C	GLY	205	-8.472	-53.215	31.265	1.000	8.34
ATOM	1545	O	GLY	205	-8.228	-54.094	30.436	1.000	9.21
ATOM	1546	N	VAL	206	-7.829	-53.130	32.425	1.000	8.74
ATOM	1547	CA	VAL	206	-6.833	-54.135	32.796	1.000	9.33
ATOM	1548	CB	VAL	206	-5.942	-53.653	33.957	1.000	16.14
ATOM	1549	CG1	VAL	206	-5.020	-54.754	34.457	1.000	6.58
ATOM	1550	CG2	VAL	206	-5.124	-52.445	33.514	1.000	6.33
ATOM	1551	C	VAL	206	-7.526	-55.447	33.154	1.000	5.34
ATOM	1552	O	VAL	206	-7.118	-56.498	32.664	1.000	5.68
ATOM	1553	N	ALA	207	-8.564	-55.384	33.982	1.000	4.56
ATOM	1554	CA	ALA	207	-9.349	-56.547	34.369	1.000	8.39
ATOM	1555	CB	ALA	207	-10.323	-56.180	35.490	1.000	0.79
ATOM	1556	C	ALA	207	-10.144	-57.160	33.219	1.000	10.03
ATOM	1557	O	ALA	207	-10.485	-58.346	33.261	1.000	13.69
ATOM	1558	N	LEU	208	-10.471	-56.382	32.193	1.000	14.72
ATOM	1559	CA	LEU	208	-11.278	-56.888	31.082	1.000	11.49
ATOM	1560	CB	LEU	208	-12.065	-55.755	30.422	1.000	12.04
ATOM	1561	CG	LEU	208	-13.325	-55.317	31.175	1.000	10.97
ATOM	1562	CD1	LEU	208	-13.985	-54.127	30.497	1.000	18.17
ATOM	1563	CD2	LEU	208	-14.302	-56.477	31.290	1.000	17.03
ATOM	1564	C	LEU	208	-10.391	-57.604	30.067	1.000	6.10
ATOM	1565	O	LEU	208	-10.857	-58.502	29.369	1.000	15.12
ATOM	1566	N	ALA	209	-9.132	-57.191	30.019	1.000	10.78
ATOM	1567	CA	ALA	209	-8.103	-57.815	29.203	1.000	16.00
ATOM	1568	CB	ALA	209	-6.827	-56.992	29.220	1.000	18.55
ATOM	1569	C	ALA	209	-7.829	-59.238	29.694	1.000	19.15
ATOM	1570	O	ALA	209	-7.639	-60.143	28.882	1.000	13.89
ATOM	1571	N	GLU	210	-7.822	-59.396	31.015	1.000	9.97
ATOM	1572	CA	GLU	210	-7.645	-60.692	31.653	1.000	11.15
ATOM	1573	CB	GLU	210	-7.535	-60.520	33.168	1.000	21.07
ATOM	1574	CG	GLU	210	-6.097	-60.365	33.647	1.000	39.63
ATOM	1575	CD	GLU	210	-5.696	-58.921	33.860	1.000	47.94
ATOM	1576	OE1	GLU	210	-5.958	-58.391	34.960	1.000	64.71
ATOM	1577	OE2	GLU	210	-5.097	-58.319	32.949	1.000	43.70
ATOM	1578	C	GLU	210	-8.791	-61.634	31.308	1.000	10.80

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ATOM    1579   O    GLU   210        -8.589 -62.787   30.927 1.000 10.93
ATOM    1580   N    GLN   211       -10.007 -61.120   31.441 1.000 10.29
ATOM    1581   CA   GLN   211       -11.190 -61.871   31.035 1.000 17.12
ATOM    1582   CB   GLN   211       -12.443 -61.052   31.363 1.000 15.73
ATOM    1583   CG   GLN   211       -12.542 -60.709   32.844 1.000 19.97
ATOM    1584   CD   GLN   211       -12.936 -61.923   33.671 1.000 20.12
ATOM    1585   OE1  GLN   211       -13.886 -62.628   33.331 1.000 17.44
ATOM    1586   NE2  GLN   211       -12.218 -62.166   34.759 1.000 12.84
ATOM    1587   C    GLN   211       -11.146 -62.237   29.556 1.000 19.66
ATOM    1588   O    GLN   211       -11.399 -63.384   29.170 1.000 12.73
ATOM    1589   N    VAL   212       -10.822 -61.287   28.679 1.000 17.48
ATOM    1590   CA   VAL   212       -10.785 -61.612   27.249 1.000 19.02
ATOM    1591   CB   VAL   212       -10.426 -60.369   26.415 1.000 14.47
ATOM    1592   CG1  VAL   212       -10.189 -60.744   24.958 1.000 15.00
ATOM    1593   CG2  VAL   212       -11.527 -59.320   26.523 1.000  8.88
ATOM    1594   C    VAL   212        -9.816 -62.745   26.936 1.000 23.29
ATOM    1595   O    VAL   212       -10.192 -63.735   26.294 1.000 25.62
ATOM    1596   N    ARG   213        -8.557 -62.645   27.361 1.000 21.16
ATOM    1597   CA   ARG   213        -7.617 -63.740   27.126 1.000 22.08
ATOM    1598   CB   ARG   213        -6.251 -63.462   27.752 1.000 19.45
ATOM    1599   CG   ARG   213        -5.577 -62.178   27.300 1.000 20.41
ATOM    1600   CD   ARG   213        -4.621 -61.690   28.380 1.000 26.40
ATOM    1601   NE   ARG   213        -3.847 -60.527   27.952 1.000 29.86
ATOM    1602   CZ   ARG   213        -3.556 -59.504   28.745 1.000 26.00
ATOM    1603   NH1  ARG   213        -3.968 -59.485   30.007 1.000 15.34
ATOM    1604   NH2  ARG   213        -2.847 -58.491   28.268 1.000 17.74
ATOM    1605   C    ARG   213        -8.157 -65.052   27.695 1.000 21.76
ATOM    1606   O    ARG   213        -7.893 -66.138   27.182 1.000 28.34
ATOM    1607   N    SER   214        -8.924 -64.952   28.780 1.000 15.76
ATOM    1608   CA   SER   214        -9.486 -66.151   29.389 1.000 15.09
ATOM    1609   CB   SER   214       -10.043 -65.824   30.781 1.000 19.35
ATOM    1610   OG   SER   214       -11.053 -66.745   31.144 1.000 46.77
ATOM    1611   C    SER   214       -10.561 -66.790   28.529 1.000 15.48
ATOM    1612   O    SER   214       -10.692 -68.016   28.535 1.000 24.87
ATOM    1613   N    LEU   215       -11.355 -66.030   27.772 1.000 21.40
ATOM    1614   CA   LEU   215       -12.367 -66.673   26.938 1.000 21.52
ATOM    1615   CB   LEU   215       -13.655 -65.855   26.860 1.000 22.40
ATOM    1616   CG   LEU   215       -14.176 -65.153   28.103 1.000 20.48
ATOM    1617   CD1  LEU   215       -15.071 -63.990   27.697 1.000 27.15
ATOM    1618   CD2  LEU   215       -14.931 -66.118   29.006 1.000 13.10
ATOM    1619   C    LEU   215       -11.884 -66.920   25.510 1.000 20.60
ATOM    1620   O    LEU   215       -12.536 -67.682   24.789 1.000 31.41
ATOM    1621   N    LEU   216       -10.790 -66.303   25.077 1.000 21.43
ATOM    1622   CA   LEU   216       -10.291 -66.503   23.718 1.000 19.55
ATOM    1623   CB   LEU   216       -10.114 -65.148   23.021 1.000 19.47
ATOM    1624   CG   LEU   216       -11.385 -64.305   22.870 1.000 16.11
ATOM    1625   CD1  LEU   216       -11.095 -63.042   22.076 1.000 17.60
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ATOM   1626  CD2 LEU   216      -12.495 -65.108  22.211 1.000  4.00
ATOM   1627  C   LEU   216       -8.983 -67.283  23.688 1.000 24.37
ATOM   1628  OT1 LEU   216       -8.472 -67.525  22.571 1.000 29.22
ATOM   1629  OT2 LEU   216       -8.463 -67.655  24.758 1.000 19.02
```

In addition to the above-described determinations, a carbamate-inhibited perhydrolase crystal was also produced and analyzed. In these experiments, a N-hexylcarbamate derivative of wild type perhydrolase was used. Wild-type perhydrolase (14.5 mg in 1 mL, 67mM NaPO4 pH 7 buffer) was titrated at room temperature with 1.25 µL aliquots of 400 mM p-nitrophenyl-N-hexylcarbamate dissolved in DMSO. Perhydrolase activity was measured with *p*-nitrophenylbutyrate assay (*See*, Example 2), as a function of time after each addition of the inhibitor. Several additions over several hours were required for complete inhibition of the enzyme. After inhibition was complete, the buffer of the inhibited enzyme solution was exchanged for 10 mM HEPES pH 8.3. This solution was stored at - 80°C until used for crystallization screening experiments were conducted as described above. The inhibitor *p*-nitrophenyl-N-hexylcarbamate was prepared by methods known in the art (*See e.g.*, Hosie *et al.*, J. Biol. Chem., 262:260-264 [1987]). Briefly, the carbamate-inhibited perhydrolase was crystallized by vapor diffusion using the hanging drop method known in the art. A ml solution of inhibited perhydrolase (15 mg/ml in 10 mM HEPES, pH 8.2), was mixed with 4 µL of a reservoir solution (30% PEG-4,000 with 0.2 M lithium sulfate and 0.1 M Tris, pH 8.5) on a plastic coverslip, then inverted and sealed for a well of 6x4 Linbro plate containing 0.5 ml of the reservoir solution and allowed to equilibrate. Crystals formed within a few days. The crystals were flash frozen in liquid nitrogen and analyzed as described above.

While the native octamer was determined in space group P4 with unit cell dimensions:

a= 98.184 b= 98.184 and c= 230.119 α=90.00 β=90.00 γ=90.00, this crystal diffracted to about 2.0 Å. The carbamate-inhibited crystal grew in the space group P1 with unit cell dimensions a=67.754, b=80.096, and c=85.974 α=104.10°, β=112.10°, and γ=97.40° and these crystals diffract to a resolution exceeding 1.0 Å.

The carbamate was bound in a manner to exploit the interactions between the keto oxygen of the carbamate and residues forming the oxyanion hole, the amide N atoms of Ser 11 and Ala 55 and Asn 94 ND2. The hydrophobic side chain extends along the hydrophobic surface of the binding site out into the surface opening between pairs of dimers in the octamer structure. The carbamate moiety direction highlights the pivotal role of the S54V mutation. The hydrophobic moiety passes adjacent to the side chain of ser 54. Mutating the serine side to valine increased the hydrophobicity, and also served as a gatekeeper to prevent hydrophilic nucleophiles (*e.g.*, water) for competing with desired deacylating nucleophiles. The t residues surrounding the carbamate moiety on the same and neighboring molecules forming the extended entry are expected to influence the selection of the optimal de-acylating nucleophile.

In addition, residues with surface-accessible side chain atoms were identified using the program "AreaMol," within the CCP4 program package. Table 15-1 lists these residues. In this Table, the residue number, residue name, number of surface-accessible side chain atoms having at least 10.0 square atoms of accessible surface area, and maximum surface area (square angstroms) for any side chain atom within that residue (or CA for GLY residues) in the octameric structure of perhydrolase are provided.

Table 15-1. Surface-Accessible Side Chain Atoms

Residue Number	Residue Name	Number of Accessible Side Chain Atoms	Maximum Surface Area (Square Angstroms)
1	ALA	1	15.7
3	LYS`	2	54.10
17	VAL	1	29.5
19	VAL	1	28.0

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20	GLU	4	30.2
21	ASP	2	41.3
24	PRO	2	23.2
26	GLU	3	36.3
29	ALA	1	34.4
30	PRP	3	32.7
31	ASP	3	50.6
32	VAL	1	27.0
39	ALA	1	27.5
40	GLN	3	38.7
41	GLN	2	22.1
43	GLY	1	20.4
44	ALA	1	63.8
45	ASP	3	52.7
46	PHE	2	17.1
47	GLU	3	29.6
61	ASP	3	53.1
63	PRO	3	28.0
64	THR	1	15.7
65	ASP	1	10.8
66	PRO	3	33.5
67	ARG	2	20.3
69	ASN	1	11.0
72	SER	2	26.6
75	PRO	2	17.4
83	PRO	2	15.1
85	ASP	1	36.80
98	ALA	1	14.60
101	ARG	4	25.0
102	ARG	1	19.9
103	THR	1	43.7
104	PRO	1	17.90
105	LEU	1	10.1
113	VAL	1	17.3
116	THR	2	39.5
117	GLN	2	15.3
119	LEU	3	21.4
120	THR	2	34.1
122	ALA	1	38.0

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123	GLY	1	11.0
126	GLY	1	11.9
128	THR	2	18.2
129	TYR	1	17.6
130	PRO	3	30.2
131	ALA	1	13.7
133	LYS	3	46.9
141	PRO	3	25.3
143	ALA	1	19.8
144	PRO	3	34.90
146	PRO	2	24.30
148	PRO	3	24.1
151	GLN	3	35.6
152	LEU	1	12.90
155	GLU	3	53.0
156	GLY	1	28.9
158	GLU	3	30.3
159	GLN	4	44.9
160	LYS	2	21.5
162	THR	2	25.0
163	GLU	2	23.3
165	ALA	1	23.1
169	SER	1	39.1
173	SER	2	33.3
174	PHE	1	11.1
175	MET	1	18.5
176	LYS	2	21.4
178	PRO	1	12.0
179	PHE	2	14.0
180	PHE	1	13.9
181	ASP	1	24.9
184	SER	1	27.0
185	VAL	1	27.5
187	SER	2	34.0
189	ASP	2	25.4
191	VAL	2	24.5
197	THR	2	21.6
198	GLU	3	43.5
199	ALA	1	50.5

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202	ARG	3	37.2
203	ASP	2	30.9
206	VAL	2	45.2
210	GLU	3	34.6
211	GLN	2	19.6
213	ARG	5	30.8
214	SER	2	20.8
215	LEU	1	25.80

EXAMPLE 16

Stain Removal

In this Example, experiments conducted to assess the stain removal abilities of perhydrolase are described.

Individual wells of 24 well culture plates were used to mimic conditions found in ordinary washing machines. Each well was filled with commercially available detergent (*e.g.*, Ariel [Procter & Gamble], WOB [AATCC], and WFK [WFK]), and pre-stained cloth discs cut to fit inside of each well were added. Temperature and agitation were accomplished by attaching the plate to the inside of a common laboratory incubator/shaker. To measure bleaching effectiveness of the perhydrolase, fabric stained with tea (EMPA # 167, available commercially from Test Fabrics) was used. A single cloth disc was placed in each well, and 1 ml of detergent liquid, containing enzyme, ester substrate, and peroxide was added. After agitation at 100 – 300 rpm @ 20 – 60°C, the fabric discs were removed, rinsed with tap water, and allowed to dry overnight. The reflectance of each individual cloth disc was measured, and plotted as an "L" value. These results are provided in Figure 21, which shows that the addition of the perhydrolase of the present invention to the detergent consistently provides a greater degree of bleaching than the detergents alone. In this Figure, "E" indicates the results for each of the detergents tested in combination with the perhydrolase of the present invention.

EXAMPLE 17

Cotton Bleaching

In this Example, experiments to assess the use of the perhydrolase of the present invention for bleaching of cotton fabrics are described.

In these experiments, six cotton swatches per canister were treated at 55°C for 60 minutes in a Launder-O-meter. The substrates used in these experiments were: 3 (3"x3") 428U and 3 (3"x3") 400U per experiments. Two different types of 100% unbleached cotton fabrics from Testfabrics were tested (style 428U (desized but not bleached army carded cotton sateen); and style 400U (desized but not bleached cotton print cloth). The liquor ratio was about 26 to 1 (~7.7 g fabric/~ 200 ml volume liquor). The perhydrolase enzyme was tested at 12.7 mgP/ml, with ethyl acetate (3 % (v/v)), hydrogen peroxide (1500 ppm), and Triton X-100 (0.001%), in a sodium phosphate buffer (100 mM) for pH 7 and pH 8; as well as in a sodium carbonate (100 mM) buffer, for pH 9 and pH 10.

Bleaching effects were quantified with total color difference by taking 4 CIE L*a*b* values per each swatch before and after the treatments using a Chroma Meter CR-200 (Minolta), and total color difference of the swatches after the treatments were calculated according to the following:

$$\text{Total color difference } (\Delta E) = \sqrt{\left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)}$$

(where ΔL, Δa, Δb, are differences in CIE L*, CIE a*, and CIE b* values respectively before and after the treatments).

Higher ΔE values indicate greater bleaching effects. The results (*See*, Figure 22) indicated that the perhydrolase showed significantly improved bleaching effects on both types of 100% cotton fabrics at pH 7 and pH 8 under the conditions tested.

It was also observed that high amounts of motes (*e.g.*, pigmented spots) disappeared on the enzyme treated substrates.

EXAMPLE 18

Linen Bleaching

In this Example, experiments conducted to assess the linen bleaching capability of the perhydrolase of the present invention are described. The same methods and conditions as describe above for cotton testing (in Example 17) were used to test linen swatches. As indicated above, experiments were conduction in a Launder-O-meter using a linen fabric (linen suiting, Style L-53; Testfabrics).

In these experiments, 3 (4"x4") linen swatches were treated with 12.7 mgP/ml of the perhydrolase enzyme with ethyl acetate (3 % v/v), hydrogen peroxide (1200 ppm), and Triton X-100 (0.001%), in a sodium phosphate buffer (100 mM) for pH 7 and pH 8. The bleaching effects were calculated as described above in Example 17. Figure 23 provides a graph showing the bleaching effects of the perhydrolase of the present invention tested at pH 7 and pH 8 on linen.

EXAMPLE 19

Detergent Compositions

In the following Example, various detergent compositions are exemplified. In these formulations, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications therein have the following meanings:

LAS	: Sodium linear C_{11-13} alkyl benzene sulfonate.
TAS	: Sodium tallow alkyl sulfate.
CxyAS	: Sodium C_{1x} - C_{1y} alkyl sulfate.
CxyEz	: C_{1x} - C_{1y} predominantly linear primary alcohol condensed with an average of z moles of ethylene oxide.
CxyAEzS	: C_{1x} - C_{1y} sodium alkyl sulfate condensed with an average of z moles of ethylene oxide. Added molecule name in the examples.
Nonionic	: Mixed ethoxylated/propoxylated fatty alcohol e.g. Plurafac LF404 being an alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5.
QAS	: $R_2.N+(CH_3)_2(C_2H_4OH)$ with $R_2 = C_{12}$-C_{14}.
Silicate	: Amorphous Sodium Silicate (SiO_2:Na_2O ratio = 1.6-3.2:1).
Metasilicate	: Sodium metasilicate (SiO_2:Na_2O ratio = 1.0).
Zeolite A	: Hydrated Aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}$. $27H_2O$
SKS-6	: Crystalline layered silicate of formula δ-$Na_2Si_2O_5$.
Sulphate	: Anhydrous sodium sulphate.
STPP	: Sodium Tripolyphosphate.
MA/AA	: Random copolymer of 4:1 acrylate/maleate, average molecular weight about 70,000-80,000.
AA	: Sodium polyacrylate polymer of average molecular weight 4,500.
Polycarboxylate	: Copolymer comprising mixture of carboxylated monomers such as acrylate, maleate and methyacrylate with a MW ranging between 2,000-80,000 such as Sokolan commercially available from BASF, being a copolymer of acrylic acid, MW4,500.
BB1	: 3-(3,4-Dihydroisoquinolinium)propane sulfonate
BB2	1-(3,4-dihydroisoquinolinium)-decane-2-sulfate
PB1	: Sodium perborate monohydrate.
PB4	: Sodium perborate tetrahydrate of nominal formula $NaBO_3.4H_2O$.
Percarbonate	: Sodium percarbonate of nominal formula $2Na_2CO_3.3H_2O_2$.
TAED	: Tetraacetyl ethylene diamine.
NOBS	: Nonanoyloxybenzene sulfonate in the form of the sodium salt.
DTPA	: Diethylene triamine pentaacetic acid.

HEDP	:	1,1-hydroxyethane diphosphonic acid.
DETPMP	:	Diethyltriamine penta (methylene) phosphonate, marketed by Monsanto under the Trade name Dequest 2060.
EDDS	:	Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer in the form of its sodium salt
Diamine	:	Dimethyl aminopropyl amine; 1,6-hezane diamine; 1,3-propane diamine; 2-methyl-1,5-pentane diamine; 1,3-pentanediamine; 1-methyl-diaminopropane.
DETBCHD		5, 12- diethyl-1,5,8,12-tetraazabicyclo [6,6,2] hexadecane, dichloride, Mn(II) salt
PAAC	:	Pentaamine acetate cobalt(III) salt.
Paraffin	:	Paraffin oil sold under the tradename Winog 70 by Wintershall.
Paraffin Sulfonate	:	A Paraffin oil or wax in which some of the hydrogen atoms have been replaced by sulfonate groups.
Aldose oxidase	:	Oxidase enzyme sold under the tradename Aldose Oxidase by Novozymes A/S
Galactose oxidase	:	Galactose oxidase from Sigma
Protease	:	Proteolytic enzyme sold under the tradename Savinase, Alcalase, Everlase by Novo Nordisk A/S, and the following from Genencor International, Inc: "Protease A" described in US RE 34,606 in Figures 1A, 1B, and 7, and at column 11, lines 11-37; "Protease B" described in US5,955,340 and US5,700,676 in Figures 1A, 1B and 5, as well as Table 1; and "Protease C" described in US6,312,936 and US 6,482,628 in Figures 1-3 [SEQ ID 3], and at column 25, line 12, "Protease D" being the variant 101G/103A/104I/159D/232V/236H/245R/248D/252K (BPN' numbering) described in WO 99/20723.
Amylase	:	Amylolytic enzyme sold under the tradename Purafact Ox Am^{R} described in WO 94/18314, WO96/05295 sold by Genencor; Natalase®, Termamyl®, Fungamyl® and Duramyl®, all available from Novozymes A/S.
Lipase	:	Lipolytic enzyme sold under the tradename Lipolase Lipolase Ultra by Novozymes A/S and Lipomax by Gist-Brocades.
Cellulase	:	Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novozymes A/S.
Pectin Lyase	:	Pectaway® and Pectawash® available from Novozymes A/S.
PVP	:	Polyvinylpyrrolidone with an average molecular weight of 60,000
PVNO	:	Polyvinylpyridine-N-Oxide, with an average molecular weight of 50,000.
PVPVI	:	Copolymer of vinylimidazole and vinylpyrrolidone, with an average molecular weight of 20,000.

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Brightener 1 : Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
Silicone antifoam : Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
Suds Suppressor : 12% Silicone/silica, 18% stearyl alcohol,70% starch in granular form.
SRP 1 : Anionically end capped poly esters.
PEG X : Polyethylene glycol, of a molecular weight of x.
PVP K60 ® : Vinylpyrrolidone homopolymer (average MW 160,000)
Jeffamine ® ED-2001 : Capped polyethylene glycol from Huntsman
Isachem ® AS : A branched alcohol alkyl sulphate from Enichem
MME PEG (2000) : Monomethyl ether polyethylene glycol (MW 2000) from Fluka Chemie AG.
DC3225C : Silicone suds suppresser, mixture of Silicone oil and Silica from Dow Corning.
TEPAE : Tetreaethylenepentaamine ethoxylate.
BTA : Benzotriazole.
Betaine : $(CH_3)_3N^+CH_2COO^-$
Sugar : Industry grade D-glucose or food grade sugar
CFAA : C_{12}-C_{14} alkyl N-methyl glucamide
TPKFA : C_{12}-C_{14} topped whole cut fatty acids.
Clay : A hydrated aluminumu silicate in a general formula $Al_2O_3SiO_2 \cdot xH_2O$. Types: Kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite.
MCAEM : Esters in the formula of $R^1O_x\,[(R^2)_m\,(R^3)_n]_p$
pH : Measured as a 1% solution in distilled water at 20°C.

EXAMPLE 20

Liquid Laundry Detergents

The following liquid laundry detergent compositions of the present invention are prepared.

	I	II	III	IV	V
LAS	18.0	-	6.0	-	-
C_{12}-C_{15} $AE_{1.8}S$	-	2.0	8.0	11.0	5.0
C_8-C_{10} propyl dimethyl amine	2.0	2.0	2.0	2.0	1.0
C_{12}-C_{14} alkyl dimethyl amine oxide	-	-	-	-	2.0

C_{12}-C_{15} AS	-	17.0	-	7.0	8.0
CFAA	-	5.0	4.0	4.0	3.0
C_{12}-C_{14} Fatty alcohol ethoxylate	12.0	6.0	1.0	1.0	1.0
C_{12}-C_{18} Fatty acid	11.0	11.0	4.0	4.0	3.0
Citric acid (anhydrous)	5.0	1.0	3.0	3.0	2.0
DETPMP	1.0	1.0	1.0	1.0	0.5
Monoethanolamine	11.0	8.0	5.0	5.0	2.0
Sodium hydroxide	1.0	1.0	2.5	1.0	1.5
Percarbonate	-	3.5	-	2.5	-
Propanediol	12.7	14.5	13.1	10.	8.0
Ethanol	1.8	1.8	4.7	5.4	1.0
Pectin Lyase	-	-	-	0.005	-
Amylase	-	0.002	-		-
Cellulase	-	-	0.0002		0.0001
Lipase	0.1	-	0.1	-	0.1
Protease A	0.05	0.3	0.055	0.5	0.2
Aldose Oxidase	0.03	-	0.3	-	0.003
PAAC	0.01	0.01	-	-	-
DETBCHD	-	-	0.02	0.01	-
SRP1	0.5	0.5	-	0.3	0.3
Boric acid	2.4	2.4	2.8	2.8	2.4
Sodium xylene sulfonate	-	-	3.0	-	-
DC 3225C	1.0	1.0	1.0	1.0	1.0
2-butyl-octanol	0.03	0.04	0.04	0.03	0.03
DTPA	0.5	0.4	0.35	0.28	0.4
Brightener 1	0.18	0.10	0.11	-	-
Perhydrolase	0.05	0.3	0.08	0.5	0.2
MCAEM (C_{12}-C_{13} $E_{6.5}$ Acetate)	3.0	8.0	12.0	1.5	4.8
Balance to 100% perfume / dye and/or water					

EXAMPLE 21

Hand-Dish Liquid Detergent Compositions

The following hand dish liquid detergent compositions of the present invention are

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prepared.

	I	II	III	IV	V	VI
C_{12}-C_{15} $AE_{1.8}S$	30.0	28.0	25.0	-	15.0	10.0
LAS	-	-	-	5.0	15.0	12.0
Paraffin Sulfonate	-	-	-	20.0	-	-
C_{10}-C_{18} Alkyl Dimethyl Amine Oxide	5.0	3.0	7.0	-	-	-
Betaine	3.0	-	1.0	3.0	1.0	-
C_{12} poly-OH fatty acid amide	-	-	-	3.0	-	1.0
C_{14} poly-OH fatty acid amide	-	1.5	-	-	-	-
$C_{11}E_9$	2.0	-	4.0	-	-	20.0
DTPA	-	-	-	-	0.2	-
Tri-sodium Citrate dihydrate	0.25	-	-	0.7	-	-
Diamine	1.0	5.0	7.0	1.0	5.0	7.0
$MgCl_2$	0.25	-	-	1.0	-	-
Protease A	0.02	0.01	0.02	0.01	0.02	0.05
Amylase	0.001	-	-	0.002	-	0.001
Aldose Oxidase	0.03	-	0.02	-	0.05	-
Sodium Cumene Sulphonate	-	-	-	2.0	1.5	3.0
PAAC	0.01	0.01	0.02	-	-	-
DETBCHD	-	-	-	0.01	0.02	0.01
PB1	1.5	2.8	1.2	-	-	-
Perhydrolase	0.02	0.01	0.03	0.01	0.02	0.05

	I	II	III	IV	V	VI
MCAEM (C_{14}-C_{15} E_7 Acetate)	3.4	2.8	4.0	2.6	4.6	6.8
Balance to 100% perfume / dye and/or water						

The pH of Compositions (I)-(VI) is about 8 to about 11

EXAMPLE 22

Liquid Automatic Dishwashing Detergent

The following liquid automatic dishwashing detergent compositions of the present are prepared.

	I	II	III	IV	V
STPP	16	16	18	16	16
Potassium Sulfate	-	10	8	-	10
1,2 propanediol	6.0	0.5	2.0	6.0	0.5
Boric Acid	4.0	3.0	3.0	4.0	3.0
$CaCl_2$ dihydrate	0.04	0.04	0.04	0.04	0.04
Nonionic	0.5	0.5	0.5	0.5	0.5
Protease B	0.03	0.03	0.03	0.03	0.03
Amylase	0.02	-	0.02	0.02	-
Aldose Oxidase	-	0.15	0.02	-	0.01
Galactose Oxidase	-	-	0.01	-	0.01
PAAC	0.01	-	-	0.01	-
DETBCHD	-	0.01	-	-	0.01
Perhydrolase	0.1	0.03	0.05	0.03	0.06
MCAEM (C_{14}-$C_{15}E_{12}$ Acetate)	5.0	3.0	12.0	8.0	1.0

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	I	II	III	IV	V
Balance to 100% perfume / dye and/or water					

EXAMPLE 23

Laundry Compositions

The following laundry compositions of present invention, which may be in the form of granules or tablet, are prepared.

	I	II	III	IV	V
Base Product					
C_{14}-C_{15}AS or TAS	8.0	5.0	3.0	3.0	3.0
LAS	8.0	-	8.0	-	7.0
C_{12}-$C_{15}AE_3S$	0.5	2.0	1.0	-	-
C_{12}-$C_{15}E_5$ or E_3	2.0	-	5.0	2.0	2.0
QAS	-	-	-	1.0	1.0
Zeolite A	20.0	18.0	11.0	-	10.0
SKS-6 (dry add)	-	-	9.0	-	-
MA/AA	2.0	2.0	2.0	-	-
AA	-	-	-	-	4.0
3Na Citrate $2H_2O$	-	2.0	-	-	-
Citric Acid (Anhydrous)	2.0	-	1.5	2.0	-
DTPA	0.2	0.2	-	-	-
EDDS	-	-	0.5	0.1	-
HEDP	-	-	0.2	0.1	-
PB1	3.0	4.8	-	-	4.0
Percarbonate	-	-	3.8	5.2	-

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	I	II	III	IV	V
NOBS	1.9	-	-	-	-
NACA OBS	-	-	2.0	-	-
TAED	0.5	2.0	2.0	5.0	1.00
BB1	0.06	-	0.34	-	0.14
BB2	-	0.14	-	0.20	-
Anhydrous Na Carbonate	15.0	18.0	8.0	15.0	15.0
Sulfate	5.0	12.0	2.0	17.0	3.0
Silicate	-	1.0	-	-	8.0
Protease B	0.033	0.033	-	-	-
Protease C	-	-	0.033	0.046	0.033
Lipase	-	0.008	-	-	-
Amylase	0.001	-	-	-	0.001
Cellulase	-	0.0014	-	-	-
Pectin Lyase	0.001	0.001	0.001	0.001	0.001
Aldose Oxidase	0.03	-	0.05	-	-
PAAC	-	0.01	-	-	0.05
Perhydrolase	0.03	0.05	1.0	0.06	0.1
MCAEM**	2.0	5.0	12.0	3.5	6.8

Balance to 100% Moisture and/or Minors*

- Perfume / Dye, Brightener / SRP1 / Na Carboxymethylcellulose/ Photobleach / $MgSO_4$ / PVPVI/ Suds suppressor /High Molecular PEG/Clay.
- ** MCAEM is selected from the group consisting of C_9-$C_{11}E_{2.5}$ Acetate, $[C_{12}H_{25}N(CH_3)(CH_2CH_2OAc)_2]^+ Cl^-$, $(CH_3)_2NCH_2CH_2OCH_2CH_2OAc$, or mixtures thereof..

EXAMPLE 24

Liquid Laundry Detergents

The following liquid laundry detergent formulations of the present invention are prepared.

	I	I	II	III	IV	V
LAS	11.5	11.5	9.0	-	4.0	-
C_{12}-$C_{15}AE_{2.85}S$	-	-	3.0	18.0	-	16.0
C_{14}-$C_{15}E_{2.5}$ S	11.5	11.5	3.0	-	16.0	-
C_{12}-$C_{13}E_9$	-	-	3.0	2.0	2.0	1.0
C_{12}-$C_{13}E_7$	3.2	3.2	-	-	-	-
CFAA	-	-	-	5.0	-	3.0
TPKFA	2.0	2.0	-	2.0	0.5	2.0
Citric Acid (Anhydrous)	3.2	3.2	0.5	1.2	2.0	1.2
Ca formate	0.1	0.1	0.06	0.1	-	-
Na formate	0.5	0.5	0.06	0.1	0.05	0.05
Na Culmene Sulfonate	4.0	4.0	1.0	3.0	1.2	-
Borate	0.6	0.6	-	3.0	2.0	3.0
Na hydroxide	6.0	6.0	2.0	3.5	4.0	3.0
Ethanol	2.0	2.0	1.0	4.0	4.0	3.0
1,2 Propanediol	3.0	3.0	2.0	8.0	8.0	5.0
Mono-ethanolamine	3.0	3.0	1.5	1.0	2.5	1.0
TEPAE	2.0	2.0	-	1.0	1.0	1.0
PB1		-	4.5	-	2.8	-
Protease A	0.03	0.03	0.01	0.03	0.02	0.02

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	I	I	II	III	IV	V
Lipase	-	-	-	0.002	-	-
Amylase	-	-	-	-	0.002	-
Cellulase	-	-	-	-	-	0.0001
Pectin Lyase	0.005	0.005	-		-	-
Aldose Oxidase	0.05	-	-	0.05	-	0.02
Galactose oxidase	-	0.04				
Perhydrolase	0.03	0.05	0.01	0.03	0.08	0.02
MCAEM (C_{12}-C_{15} E_6 Acetate)	3.2	4.6	1.8	3.5	6.2	2.8
PAAC	0.03	0.03	0.02	-	-	-
DETBCHD	-	-	-	0.02	0.01	-
SRP 1	0.2	0.2	-	0.1	-	-
DTPA	-	-	-	0.3	-	-
PVNO	-	-	-	0.3	-	0.2
Brightener 1	0.2	0.2	0.07	0.1	-	-
Silicone antifoam	0.04	0.04	0.02	0.1	0.1	0.1

Balance to 100% perfume / dye, and/or water

EXAMPLE 25

Compact High-Density Dishwashing Detergents

The following compact high density dishwashing detergent of the present invention are prepared:

	I	II	III	IV	V	VI
STPP	-	45.0	45.0	-	-	40.0

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	I	II	III	IV	V	VI
3Na Citrate $2H_2O$	17.0	-	-	50.0	40.2	-
Na Carbonate	17.5	14.0	20.0	-	8.0	33.6
Bicarbonate	-	-	-	26.0	-	-
Silicate	15.0	15.0	8.0	-	25.0	3.6
Metasilicate	2.5	4.5	4.5	-	-	-
PB1	-	-	4.5	-	-	-
PB4	-	-	-	5.0	-	-
Percarbonate	-	-	-	-	-	4.8
BB1	-	0.1	0.1	-	0.5	-
BB2	0.2	0.05	-	0.1	-	0.6
Nonionic	2.0	1.5	1.5	3.0	1.9	5.9
HEDP	1.0	-	-	-	-	-
DETPMP	0.6	-	-	-	-	-
PAAC	0.03	0.05	0.02	-	-	-
Paraffin	0.5	0.4	0.4	0.6	-	-
Protease B	0.072	0.053	0.053	0.026	0.059	0.01
Amylase	0.012	-	0.012	-	0.021	0.006
Lipase	-	0.001	-	0.005	-	-
Pectin Lyase	0.001	0.001	0.001	-	-	-
Aldose Oxidase	0.05	0.05	0.03	0.01	0.02	0.01
Perhydrolase	0.072	0.053	0.053	0.026	0.059	0.01
MCAEM (C_{12}-C_{13} $E_{6.5}$ Acetate)	3.5	2.8	1.6	7.5	4.2	0.8
BTA	0.3	0.2	0.2	0.3	0.3	0.3
Polycarboxylate	6.0	-	-	-	4.0	0.9

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	I	II	III	IV	V	VI
Perfume	0.2	0.1	0.1	0.2	0.2	0.2

Balance to 100% Moisture and/or Minors*

*Brightener / Dye / SRP1 / Na Carboxymethylcellulose/ Photobleach / $MgSO_4$ / PVPVI/ Suds suppressor /High Molecular PEG/Clay.

The pH of compositions (I) through (VI) is from about 9.6 to about 11.3.

EXAMPLE 26

Tablet Detergent Compositions

The following tablet detergent compositions of the present invention are prepared by compression of a granular dishwashing detergent composition at a pressure of 13KN/cm^2 using a standard 12 head rotary press.

	I	II	III	IV	V	VI	VII	VIII
STPP	-	48.8	44.7	38.2	-	42.4	46.1	36.0
3Na Citrate $2H_2O$	20.0	-	-	-	35.9	-	-	-
Na Carbonate	20.0	5.0	14.0	15.4	8.0	23.0	20.0	28.0
Silicate	15.0	14.8	15.0	12.6	23.4	2.9	4.3	4.2
Lipase	0.001	-	0.01	-	0.02	-	-	-
Protease B	0.042	0.072	0.042	0.031	-	-	-	-
Protease C	-	-	-	-	0.052	0.023	0.023	0.029
Perhydrolase	0.01	0.08	0.05	0.04	0.052	0.023	0.023	0.029
MCAEM (C_{12}-C_{13} $E_{6.5}$ Acetate)	2.8	6.5	4.5	3.8	4.6	2.8	2.8	2.8
Amylase	0.012	0.012	0.012	-	0.015	-	0.017	0.002

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	I	II	III	IV	V	VI	VII	VIII
Pectin Lyase	0.005	-	-	0.002	-	-	-	-
Aldose Oxidase	-	0.03	-	0.02	0.02	-	0.03	-
PB1	-	-	3.8	-	7.8	-	-	8.5
Percarbonate	6.0	-	-	6.0	-	5.0	-	-
BB1	0.2	-	0.5	-	0.3	0.2	-	-
BB2	-	0.2	-	0.5	-	-	0.1	0.2
Nonionic	1.5	2.0	2.0	2.2	1.0	4.2	4.0	6.5
PAAC	0.01	0.01	0.02	-	-	-	-	-
DETBCHD	-	-	-	0.02	0.02	-	-	-
TAED	-	-	-	-	-	2.1	-	1.6
HEDP	1.0	-	-	0.9	-	0.4	0.2	-
DETPMP	0.7	-	-	-	-	-	-	-
Paraffin	0.4	0.5	0.5	0.5	-	-	0.5	-
BTA	0.2	0.3	0.3	0.3	0.3	0.3	0.3	-
Polycarboxylate	4.0	-	-	-	4.9	0.6	0.8	-
PEG 400-30,000	-	-	-	-	-	2.0	-	2.0
Glycerol	-	-	-	-	-	0.4	-	0.5
Perfume	-	-	-	0.05	0.2	0.2	0.2	0.2

Balance to 100% Moisture and/or Minors*

*Brightener / Dye / SRP1 / Na Carboxymethylcellulose/ Photobleach / $MgSO_4$ / PVPVI/ Suds suppressor /High Molecular PEG/Clay.

The pH of Compositions (I) through 7(VIII) is from about 10 to about 11.5.

The tablet weight of Compositions 7(I) through 7(VIII) is from about 20 grams to about 30 grams.

EXAMPLE 27

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Liquid Hard Surface Cleaning Detergents

The following liquid hard surface cleaning detergent compositions of the present invention are prepared.

	I	II	III	IV	V	VI	VII
C_9-$C_{11}E_5$	2.4	1.9	2.5	2.5	2.5	2.4	2.5
C_{12}-$C_{14}E_5$	3.6	2.9	2.5	2.5	2.5	3.6	2.5
C_7-C_9E_6	-	-	-	-	8.0	-	-
C_{12}-$C_{14}E_{21}$	1.0	0.8	4.0	2.0	2.0	1.0	2.0
LAS	-	-	-	0.8	0.8	-	0.8
Sodium culmene sulfonate	1.5	2.6	-	1.5	1.5	1.5	1.5
Isachem ® AS	0.6	0.6	-	-	-	0.6	-
Na_2CO_3	0.6	0.13	0.6	0.1	0.2	0.6	0.2
3Na Citrate $2H_2O$	0.5	0.56	0.5	0.6	0.75	0.5	0.75
NaOH	0.3	0.33	0.3	0.3	0.5	0.3	0.5
Fatty Acid	0.6	0.13	0.6	0.1	0.4	0.6	0.4
2-butyl octanol	0.3	0.3	-	0.3	0.3	0.3	0.3
PEG DME-2000®	0.4	-	0.3	0.35	0.5	-	-
PVP	0.3	0.4	0.6	0.3	0.5	-	-
MME PEG (2000) ®	-	-	-	-	-	0.5	0.5
Jeffamine ® ED-2001	-	0.4	-	-	0.5	-	-
PAAC	-	-	-	0.03	0.03	0.03	-
DETBCHD	0.03	0.05	0.05	-	-	-	-
Protease B	0.07	0.05	0.05	0.03	0.06	0.01	0.04
Amylase	0.12	0.01	0.01	-	0.02	-	0.01
Lipase	-	0.001	-	0.005	-	0.005	-
Perhydrolase	0.07	0.05	0.08	0.03	0.06	0.01	0.04

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	I	II	III	IV	V	VI	VII
MCAEM (C_{12}-$C_{15}E_8$ Acetate)	3.5	5.6	4.8	5.3	3.6	8.0	4.7
Pectin Lyase	0.001	-	0.001	-	-	-	0.002
PB1	-	4.6	-	3.8	-	-	-
Aldose Oxidase	0.05	-	0.03	-	0.02	0.02	0.05

Balance to 100% perfume / dye, and/or water

The pH of Compositions (I) through (VII) is from about 7.4 to about 9.5.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

Those of skill in the art readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The compositions and methods described herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It is readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

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CLAIMS

What is claimed is:

1. An isolated perhydrolase, wherein said perhydrolase exhibits a perhydrolysis to hydrolysis ratio that is greater than 1.

2. The perhydrolase of Claim 1, wherein said perhydrolase is *M. smegmatis* perhydrolase.

3. An isolated perhydrolase, wherein said perhydrolase is at least approximately about 35% homologous to said *M. smegmatis* perhydrolase of Claim 2.

4. The perhydrolase of Claim 2, wherein said perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2.

5. An isolated perhydrolase having immunological cross-reactivity with said perhydrolase of Claim 2.

6. The perhydrolase of Claim 2, wherein said perhydrolase is at least a portion of said *M. smegmatis* perhydrolase, wherein said perhydrolase has a perhydrolysis to hydrolysis ration that is greater than 1.

7. The perhydrolase of Claim 2, wherein said perhydrolase is a structural homologue of said *M. smegmatis* perhydrolase, in which the active site is homologous to at least one amino acid selected from the group consisting of S11, D192, and H195 of the *M. smegmatis* perhydrolase.

8. An isolated perhydrolase variant having an amino acid sequence comprising at least one modification of an amino acid made at a position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2.

9. The perhydrolase variant of Claim 8, wherein said at least one modification is made at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said modified amino acid is selected from the group consisting of Cys7, Asp10, Ser11, Leu12, Thr13, Trp14, Trp16, Pro24, Thr25, Leu53, Ser54, Ala55, Thr64, Asp65, Arg67, Cys77, Thr91, Asn94, Asp95, Tyr99, Val125, Pro138, Leu140, Pro146, Pro148, Trp149, Phe150, Ile153, Phe154, Thr159, Thr186, Ile192, Ile194, and Phe196.

10. The perhydrolase variant of Claim 8, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of M1, K3, R4, I5, L6, C7, D10, S11, L12, T13, W14, W16, G15, V17, P18, V19, D21, G22, A23, P24, T25, E26, R27, F28, A29, P30, D31, V32, R33, W34, T35, G36, L38, Q40, Q41, D45, L42, G43, A44, F46, E47, V48, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, I60, D61, D62, P63, T64, D65, P66, R67, L68, N69, G70, A71, S72, Y73, S76, C77, L78, A79, T80, L82, P83, L84, D85, L86, V87, N94, D95, T96, K97, Y99F100, R101, R102, P104, L105, D106, I107, A108, L109, G110, M111, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P146, P148, W149, F150, I153, F154, I194, and F196.

11. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in peracid hydrolysis compared to the wild-type perhydrolase.

12. The perhydrolase variant of Claim 11, wherein said change in peracid hydrolysis is a decrease.

13. The perhydrolase variant of Claim 11, wherein said change in peracid hydrolysis is an increase.

14. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.1 or less, in comparison with wild-type perhydrolase.

15. The perhydrolase variant of Claim 14, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of R4, L12, G15, P18, R27, W34L38, A44, E51, G52, L53, S54, T58, R67, L68, S72, A79, T80, D85, L86, V87, N94, K97, R101, V118, L119, G124, G126, and I194.

16. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.2 or less, in comparison with wild-type perhydrolase.

17. The perhydrolase variant of Claim 16, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID

NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, P18, V19, T25, R27, W34, L38, A44, I49, E50, E51, G52, L53, S54, A55, R56, T58, N59, D62, T64, D65, R67, L68, N69, S72, S76, C77, A79, T80, D85, L86, V87, N94, K97, R101, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, V118, L119, A122, G124, G126, T127, Y129, W149, and I194.

18. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.3 or less, in comparison with wild-type perhydrolase.

19. The perhydrolase variant of Claim 18, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, D10, L12, W14, G15, L12, P18, V19, G22, A23, T25, E26, R27, W34, G36, L38, Q41, L42, G43, A44, I49, E50, E51, G52, L53, S54, A55, R56, T57, N59, T58, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, T96, K97, Y99, F100, R101, R102, P104, L109, G110, M111, L114, V118, L119, A122, G124, V125, G126, T127, Y129, W149, F154, and I194.

20. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.4 or less, in comparison with wild-type perhydrolase.

21 The perhydrolase variant of Claim 20, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID

NO:2, wherein said at least one substitution is selected from the group consisting of R4, I5, L6, D10, S11, L12, W14, G15, W16, P18, V19, G22, A23, T25, E26, R27, F28, W34, T35, G36, L38, Q41, L42, G43, A44, D45, E47, I49, E50, E51, G52, L53, S54, A55, R56, T57, T58, N59, T58, I60, D62, T64, D65, R67, L68, N69, G70, S72, Y73, S76, C77, A79, T80, L82, P83, D85, L86, V87, N94, P66, T96, K97, Y99, F100, R101, R102, P104, I107, L109, G110, M111, S112, L114, V118, L119, S121, A122, G124, V125, G126, T127, Y129, W149, F150,.F154, I194, and F196.

22. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.5 or less, in comparison with wild-type perhydrolase.

23. The perhydrolase variant of Claim 22, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109,

L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

24. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.6 or less, in comparison with wild-type perhydrolase.

25. The perhydrolase variant of Claim 24, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194,

K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, V125, V19, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

26. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.7 or less, in comparison with wild-type perhydrolase.

27. The perhydrolase variant of Claim 26, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106,

D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y73, Y99, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

28. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 0.8 or less, in comparison with wild-type perhydrolase.

29. The perhydrolase variant of Claim 28, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109, L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y1d29, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85,

E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95, E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89, L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117, R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19, V32, V87, W149, Y129, Y73, G190, V191, G193, T197, N201, D203, L208, A209, V212, L215, and L216.

30. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis of about 1.5 or greater, in comparison with wild-type perhydrolase.

31. The perhydrolase variant of Claim 30, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A122, A23, A29, A55, D45, D62, D65, E26, E50, F150, F46, G110, G124, G43, L109,

L119, L42, L68, L78, L82, L84, N59, P66, R101, R27, R4, R67, S112, S54, S76, T116, T120, T25, V125, V48, W149, Y73, A44, A79, D85, E51, G124, G126, G15, G52, I194, K97, L119, L12, L38, L53, L68, L86, N94, P18, R101, R27, R4, R67, S54, S72, T58, T80, V118, V87, W34, R4, I5, D10, L12, W14, V19, T25, W34, I49, E50, E51, L53, S54, A55, R56, N59, D62, T64, D65, R67, L68, N69, S76, C77, T80, L82, P83, L86, V87, N94, T96, F100, R101, L109, M111, L114, L119, W149, Y129, A122, G126, T127, A23, A55, A79, D65, D85, E26, F154, G110, G124, G126, G22, G36, G43, G52, G70, I49, K97, L109, L114, L119, L12, L38, L42, L53, L68, L86, P104, P83, Q41, R102, R56, R67, S54, T57, V118, V125, W14, W149, Y129, Y73, A122, A23, A79, D45, D65, D85, E26, E47, E51, F150, F196, F28, G110, G124, G36, G43, G52, G70, I107, I5, I60, L109, L119, L53, L6, L68, L82, M111, P104, P66, R102, R67, S11, S112, S121, S54, S72, T25, T35, T57, T58, V118, V125, V19, W149, W16, A108, A122, A23, A29, A79, C7, D106, D21, D45, D62, D65, D85, E50, F150, F28, G124, G126, G22, G36, G52, I107, I194, K97, L105, L109, L114, L119, L38, L68, L78, L82, L84, M111, N69, N94, P104, P63, P66, R102, R27, S11, S112, S54, S72, T116, T120, T127, T13, T25, T57, T80, T96, V113, A122, A29, A71, A79, C7, D106, D21, D61, D65, D85, E47, E50, F150, F196, F28, F46, G124, G126, G15, G36, G70, I49, I5, I60, L105, L109, L12, L38, L42, L53, L84, L86, M111, N59, P146, P24, P66, Q41, R102, R27, R56, S112, S121, S54, S72, T116, T120, T127, T128, T13, T57, T64, V125, V17, V19, W14, W149, W16, Y129, Y99, A108, A122, A23, A29, A44, A55, A71, A79, C77, D45, D61, D65, D85, D95, E47, E51, F150, F196, F46, G110, G126, G36, G43, G52, I107, I194, I49, I5, I60, I89, L114, L42, L53, L68, L78, L84, M111, N59, N94, P146, P24, P30, P63, P66, P83, Q117, R101, R4, S112, S121, S72, T116, T120, T127, T13, T57, T96, V113, V125, V17, V19, V32, V87, W149, Y129, and Y73, Y99, A108, A44, C7, D10, D106, D31, D61, D85, E26, E51, F100, F28, F46, G110, G22, G36, G43, G52, G70, I107, I153, I49, I5, I89, K3, L105, L53, L6, L78, L86, M1, N69, P104, P146, P18, P24, P30, P83, Q117, Q40, Q41, R102, R27, R33, R4, S121, S72, S76, T120, T128, T13, T35, T80, T96, V115, V118,

V32V48, V87, W34, G190, V191, G193, T197, E198, A199, R202, D203, G205, V206, A209, E210, Q211, S214, and L215.

32. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a ratio of peracid hydrolysis between about 1.2 and about 1.5, in comparison with wild-type perhydrolase.

33. The perhydrolase variant of Claim 32, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, C7, D106, D31, D61, D85, E26, E50, E51, F100, F150, F28, F46, G110, G126, G22, G70, I107, K3, L105, L42, L6, L78, M111, N59, N69, P104, P146, P148, P18, P30, P63, Q117, Q40, Q41, R102, R27, R33, R4, S54, S76, T116, T120, T128, T64, T80, T96, V113, V115, V118, W34, and Y73.

34. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is at least about 1.2.

35. The perhydrolase variant of Claim 34, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of C7, D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41, L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66,

R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97, R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124, V125, G126, T127, P148, F150, I153, F154, and F196.

36. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less.

37. The perhydrolase variant of Claim 36, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A108, A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D45, D61, D62, D65, D85, E26, E47, E50, E51, F100, F150, F154, F196, F28, F46, G110, G124, G126, G15, G22, G36, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105, L109, L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, K86, M1, M111, N59N94, P146, P18, P24, P30, P66, P83, Q40, Q41, R101, R102, R27, R33, R4, R56, R67, S11, S112, S54, S72, S76, T103, T116, T120, T127, T128, T13, T25, T35, T57, T64, T80, T96, V113, V115, V118, V125, V17, V19, V32, V48, V87, W13, W149, W16, W34, Y129, Y73, and Y99.

38. The perhydrolase variant of Claim 8, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A108, A122, A23, A29, A44, A55, A71, A79, C7, C77, D10, D106, D21, D31, D45, D61, D62, D65, D85, E26, E47, E50, E51, F100, F150, F154F196, F28, F46, G110, G124, G126,

G15, G22, G36, G43, G52, G70, I107, I153, I194, I49, I5, I60, I89, K3, K97, L105, L109, L114, L119, L12, L38, L42, L53, L6, L68, L78, L82, L84, L86, M1, M111, N59, N69, N94, P104, P146, P148, P18, P24, P30, P63, P66, P83, Q117, Q40, Q41, R101, R102, R27, R33, R4, R56, R67, S11, S112, S121, S54, S72, S76, T103, T116, T120, T127, T128, T13, T25, T35, T57, T58, T64, T80, T96, V113, V115, V118, V125, V17, V19, V32, V48, V87, W14, W149, W16, W34, Y129, Y73, and Y99.

39. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 1.2 and about 2.

40. The perhydrolase variant of Claim 39, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of C7, D10, L12, G15, P18, V19, G22, T25, E26, R27, F28, A29, P30, D31, G36, Q40, Q41, L42, G43, A44, D45, F46, E47, I49, E51, L53, S54, A55, T57, D61, P63, T64, D65, P66, R67, L68, N69, A71, S72, Y73, S76, L78, A79, T80, L82, P83, D85, L86, D95, K97, R101, T103, P104, L105, D106, I107, L109, M111, V113, Q117, V118, S121, G124, V125, G126, T127, P148, F150, I153, F154, F196, G190, E198, A199, R202, D203, V206, A209, E210, Q211, and V212.

41. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2 and about 2.5.

42. The perhydrolase variant of Claim 41, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A44, C7, D10, D85, D95, E26, E47, I107, L12, L42, P104, P148, S54, Q40, Q117, D203, V206, E210.

43. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 2.5 and about 3.

44. The perhydrolase variant of Claim 43, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A44, C7, I107, K97, L12, L78, P104, Q40, and V125.

45. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is between about 3.0 and about 5.

46. The perhydrolase variant of Claim 45, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of D10, D85, L53, L78, and S54.

47. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.1 or less.

48. The perhydrolase variant of Claim 47, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, and W34.

49. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.2 or less.

50. The perhydrolase variant of Claim 49, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, and Y73.

51. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.3 or less.

52. The perhydrolase variant of Claim 51, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, and Y129.

53. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.4 or less.

54. The perhydrolase variant of Claim 53, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6,

L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, and V87.

55. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.5 or less.

56. The perhydrolase variant of Claim 55, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77,

E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, and Y129 .

57. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.6 or less.

58. The perhydrolase variant of Claim 57, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24,

Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149, and Y73.

59. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.7 or less.

60. The perhydrolase variant of Claim 59, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36, I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115,

V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149,Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, and Y99.

61. The perhydrolase variant of Claim 8, wherein said variant perhydrolase exhibits a change in perhydrolysis, such that the ratio of variant perhydrolase perhydrolysis to wild-type perhydrolase perhydrolysis is about 0.8 or less.

62. The perhydrolase variant of Claim 36, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A23, A55, D10, D62, F150, F196, F28, G110, G52, G70, I107, I194, I5, K97, L12, L53, L6, L86, N94, P83, R102, R4, R56, S11, S54, T120, T13, T25, T80, V115, V19, V32, V48, V87, W14, W149, W16, W34, A108, A23, A55, D62, F150, F154, G110, G22, G52, G70, I194, K3, K97, L105, L12, L38, L53, L68, L84, N59, N94, P146, P18, R102, R33, R4, R56, S112, S54, T127, T13, T35, T64, T80, T96, V118, V48, W149, W16, W34, Y129, Y73, A122, A23, A44, C7, D10, D62, F150, G110, G22, G70, I153, I194, I60, I89, K97, L114, L119, L12, L38, L6, L68, L82, M111, N94, P146, Q41, R102, R27, R4, R56, S11, S54, T120, T13, T25, T35, T80, V48, W14, W149, W16, W34, Y129, A55, C77, E51, F100, F150, F154, G110, G126, G22, I194, I89, K97, L114, L84, N59, P146, P83, R102, R27, R33, R4, R56, S112, S54, S72, S76, T120, T127, T13, T25, T57, T96, V118, V125, V19, V87, A23, A55, D10, D23, E26, E50, E51, F150, G110, G126, G15, G36,

I107, I49, I5, K97, L109 ,L119, L12 L38, L6, L68, L84, L86, M111, N59, P146, P24, Q40, R101, R102, R27, R33, R4, R56, S112, S72, S76, T127, T25, T35, T80, T96, V115, V32, V87, W34, Y129, A108, A44, A55, D21, D62, F150, g126, G36, G52, I107, I5, I89, L109, L114, L119, L12, L42, L53, L6, L68, L78, L84, P146, P24, P66, P83, R27, S112, S72, S76, T120, T127, T13, T35, T57, T58, T80, T96, V115,V118, V32, V48, V87, W149,Y73, A122, A23, A29, A71, A79, C7, D61, D62, D85, E26, E51, F100, F28, F46, G110, G126, G52, G70, I107, I49, I5, I60, I89, L109, L114, L12, L38, L68, L82, L86, M111, N59, N94, P83, R102, R33, R4, S112, S72, S76, T103, T116, T128, T25, T35, T57, T58, T64, V19, V32, V48, V87, Y129, Y73, Y99, A108, A122, A29, A55, C77, D10, D106, D45, D61, D62, D65, D85, E47, E50, F100, F150, F28, F46, G110, G124, G126, G15, G36, I153, I194, I5, I60, I89, K3, K97, L105, L109, L114, L119, L38, L42, L68, L84, L86, M1, N59, P24, P30, P83, R101, R27, R4, R56, S112, S54, S76, T103, T116, T120, T127, T128, T13, T35, T64, V113, V17, V19, V32, V48, V87, Y129, Y73, and Y99.

63. A perhydrolase variant, wherein said variant exhibits greater perhydrolysis activity and decreased peracid hydrolysis activity as compared to wild-type perhydrolase.

64. The perhydrolase variant of Claim 8, wherein said perhydrolase exhibits perhydrolysis activity ratio of at least about 1.2, and peracid hydrolysis activity ratio of about 0.8 or less, as compared to wild-type perhydrolase.

65. The perhydrolase variant of Claim 64, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A29, A44, A55, A71, A79, C7, D10, D106, D31, D85, E26, E47, F150, F154, F196, F28,

G124, G126, G36, G43, I153, L109, L42, L53, L109, L42, L53, L109, L42, L53, L68, L82, L86, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S54, S121, S72, S76, T25, T64, V115, and V19.

66. The perhydrolase variant of Claim 8, wherein said perhydrolase exhibits perhydrolysis activity ratio of at least about 1.2, a peracid hydrolysis activity ratio of about 0.8 or less, and a protein concentration ratio of at least 0.5, as compared to wild-type perhydrolase.

67. The perhydrolase variant of Claim 66, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A29, A44, A71, A79, C7, D85, E26, E47, E51, F150, F154, F196, F28, G124, G126, G36, I153, L109, L12, L53, L68, L82, M111, N69, P104, P148, P18, P63, P66, P83, Q117, Q40, R101, R67, S121, S54, S72, S76, T25, T64, V125, and V19.

68. A perhydrolase variant wherein said variant perhydrolase exhibits an increase in expression of said perhydrolase variant, as compared to the expression of wild-type perhydrolase.

69. The perhydrolase variant of Claim 68, wherein said modification comprises at least one substitution at an amino acid position equivalent to a position in *M. smegmatis* perhydrolase comprising the amino acid sequence set forth in SEQ ID NO:2, wherein said at least one substitution is selected from the group consisting of A2, I5, C7, F8, S11, L12, T13, W14, W16, V17, P18, V19, E20, G22, A23, P24, T25, A29, P30, V32, T35, G36, V37, A39, F46, E47, S54, A55, R56, T58, I60, D61, D62, P63, T64,

P66, R67, L68, N69, G70, S72, Y73, L74, P75, S76, C77, L78, A79, T80, L82, P83, L84, L86, I89, T93, T96, K97, A98, Y99, F100, R101, R102, T103, P104, L105, D106, I107, A108, L109, G110, S112, V113, L114, V115, T116, Q117, V118, L119, T120, S121, A122, G124, V125, G126, T127, T128, Y129, P130, P132, K133, L135, V136, S138, P141, L142, A143, M145, H147, W149, F150, Q151, I153, G157, Q159, T161, T162, L164, A165, R166, V167, Y168, A170, L171, A172, M175, K176, P178, A182, G183, S184, V185, I186, T188, I194, F196, V191, N201, L208, A209, Q211, Q213, S214, L215, and L216.

70. An isolated protein comprising a homolog of *M. smegmatis* perhydrolase, wherein said homolog is a protein within the SGNH-hydrolase family of proteins.

71. An isolated protein having at least about 35% identity with the amino acid sequence of *M. smegmatis* perhydrolase, in which the protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195.

72. An isolated protein having at least about 38% identity with the amino acid sequence of *M. smegmatis* perhydrolase, wherein said protein exhibits perhydrolysis activity.

73. A homolog of *M. smegmatis* perhydrolase, wherein said homolog is a perhydrolase comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.

74. The homolog of Claim 72, wherein said homolog exhibits perhydrolysis.

75. The homolog of Claim 72, wherein said homolog exhibits a perhydrolysis to hydrolysis ratio that is great than about 1.

76. The homolog of Claim 72, wherein said homolog is immunologically cross-reactive with antibodies raised against said *M. smegmatis* perhydrolase.

77. The homolog of Claim 72, wherein antibodies raised against said homolog cross-react with said *M. smegmatis* perhydrolase.

78. An isolated protein having at least about 35% identity with the amino acid sequence of at least one *M. smegmatis* perhydrolase homolog, wherein said protein exhibits perhydrolysis activity.

79. An isolated protein having perhydrolase activity, wherein said protein is in the form of a multimer in solution.

80. The isolated protein of Claim 79, wherein said protein is a perhydrolase that comprises a dimer.

81. The isolated protein of Claim 79, wherein said protein is a perhydrolase that comprises an octamer.

82. An isolated protein having perhydrolase activity, wherein said protein is in the form of a multimer in solution and said protein is selected from the group consisting of *M. smegmatis* perhydrolase, *M. smegmatis* perhydrolase homologs, and *M. smegmatis* perhydrolase variants.

83. The isolated protein of Claim 79, wherein said protein is selected from the group consisting of modified serine hydrolases and modified cysteine hydrolases, wherein said modified serine hydrolases or said modified cysteine hydrolases comprise increased perhydrolase activity as compared to unmodified serine hydrolases or unmodified cysteine hydrolases

84. An isolated protein having perhydrolase activity, wherein said protein comprises at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.

85. The isolated protein of Claim 84, wherein said protein is obtained from a member of the *Rhizobiales.*

86. The isolated protein of Claim 84, wherein said protein is obtained from a member of the genus *Mycobacterium.*

87. An isolated gene identified using at least one primer selected from the group consisting of SEQ ID NOS:21-69.

88. A method for identifying a perhydrolase, comprising the steps of:

a) identifying source of said perhydrolase;

b) analyzing said source to identify sequences comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT;

c) expressing said sequences identified in step b) to produce said perhydrolase; and

d) testing said perhydrolase for perhydrolysis activity.

89. The method of Claim 88, wherein said analyzing step is an amplification step wherein the primer sequences set forth in SEQ ID NOS:21-69 are used to amplifying said sequences comprising at least one motif selected from the group consisting of GDSL-GRTT, GDSL-ARTT, GDSN-GRTT, GDSN-ARTT, and SDSL-GRTT.

90. The method of Claim 88, wherein said source is selected from the group consisting of environmental sources and metagenomic sources.

91. The protein identified using the method of Claim 88.

92. An isolated nucleic acid sequence encoding the protein of Claim 91.

93. The method of Claim 88, wherein said protein exhibits a perhydrolysis to hydrolysis ratio that is greater than about 1.

94. The method of Claim 88, wherein said protein exhibits a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase.

95. The method of Claim 88, wherein said protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195.

96. The method of Claim 88, wherein said analyzing step comprises searching at least one amino acid database.

97. The method of Claim 88, wherein said analyzing step comprises searching at least one nucleic acid database to identify nucleic acid sequences encoding the amino acid sequences of said perhydrolase.

98. The method of Claim 96, wherein said source is selected from the group consisting of environmental sources and metagenomic sources.

99. An isolated nucleic acid sequence encoding the protein of Claim 96.

100. An isolated nucleic acid sequence identified using the method of Claim 96.

101. The method of Claim 96, wherein said protein exhibits a perhydrolysis to hydrolysis ratio that is greater than about 1.

102. The method of Claim 96, wherein said protein exhibits a perhydrolysis activity that is at least about 0.2, compared to the perhydrolysis activity exhibited by *M. smegmatis* perhydrolase.

103. The method of Claim 96, wherein said protein comprises at least three residues selected from the group consisting of L6, W14, W34, L38, R56, D62, L74, L78, H81, P83, M90, K97, G110, L114, L135, F180, G205, S11, D192, and H195, as set forth in SEQ ID NO:2.

104. A variant perhydrolase of Claim 1, wherein said variant perhydrolase has an altered substrate specificity as compared to wild-type *M. smegmatis* perhydrolase.

105. The variant perhydrolase of Claim 104, wherein said variant has altered para nitrophenyl caproate (PNC) activity, as compared to wild-type *M. smegmatis* perhydrolase.

106. A variant perhydrolase of Claim 1, wherein said variant perhydrolase has an altered pI as compared to wild-type *M. smegmatis* perhydrolase.

107. The variant perhydrolase of Claim 106, wherein said variant perhydrolase comprises at least one positively charged mutation.

108. The variant perhydrolase of Claim 106, wherein said variant perhydrolase comprises at least one negatively charged mutation.

109. A variant perhydrolase of Claim 1, wherein said variant has increased stability, as compared to wild-type *M. smegmatis* perhydrolase.

110. The variant perhydrolase of Claim 109, wherein said stability is selected from the group consisting of thermostability, enzymatic stability, and chemical stability.

111. The perhydrolase of Claim 1, wherein said variant exhibits at least one altered surface property.

112. The perhydrolase of Claim 111, wherein said variant comprises mutations at least one substitution at sites selected from the group consisting of the residues set forth in Table 15-1.

113. The perhydrolase of Claim 1, wherein said perhydrolase is a variant

perhydrolase having at least one improved property as compared to wild-type perhydrolase.

114. An expression vector comprising a polynucleotide sequence encoding the perhydrolase variant of Claim113.

115. A host cell comprising said expression vector of Claim 114.

116. The host cell of Claim 115, wherein said host is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp.

117. A perhydrolase produced by said host cell of Claim 116.

118. A composition comprising at least a portion of the isolated perhydrolase of Claim 1.

119. The composition of Claim 118, wherein said perhydrolase comprises the amino acid sequence set forth in SEQ ID NO:2.

120. The composition of Claim 118, wherein said perhydrolase is encoded by the polynucleotide sequence set forth in SEQ ID NO:1.

121. The polynucleotide sequence of Claim 1218, wherein said sequence comprises at least a portion of SEQ ID NO:1.

122. An expression vector comprising the polynucleotide sequence of Claim 121.

123. A host cell comprising said expression vector of Claim 122.

124. The host cell of Claim 123, wherein said host is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Escherichia*, and *Pantoea* sp.

125. A perhydrolase produced by said host cell of Claim 124.

126. A variant perhydrolase, wherein said perhydrolase comprises at least one substitution corresponding to the amino acid positions in SEQ ID NO:2, and wherein said variant perhydrolase has better performance in at least one property, compared to wild-type *M. smegmatis* perhydrolase.

127. An isolated polynucleotide comprising a nucleotide sequence (i) having at least about 70% identity to SEQ ID NO:1, or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence set forth in SEQ ID NO:1, under conditions of intermediate to high stringency, or (iii) being complementary to the nucleotide sequence set forth in SEQ ID NO:1.

128. A vector comprising the polynucleotide of Claim 127.

129. A host cell transformed with the vector of Claim 128.

130. A polynucleotide comprising a sequence complementary to at least a portion of the sequence set forth in SEQ ID NO:1.

131. A method of producing an enzyme having perhydrolase activity,

comprising:

(a) transforming a host cell with an expression vector comprising a polynucleotide having at least 70% sequence identity to SEQ ID NO:1;

(b) cultivating said transformed host cell under conditions suitable for said host cell to produce said perhydrolase; and

(c) recovering said perhydrolase.

132. The method of Claim 131, wherein said host cell is selected from the group consisting of *Streptomyces*, *Pantoea*, *Escherichia*, and *Bacillus* species.

133. A probe comprising a 4 to 150 polynucleotide sequence substantially identical to a corresponding fragment of SEQ ID NO:1, wherein said probe is used to detect a nucleic acid sequence coding for an enzyme having perhydrolase activity.

134. A cleaning composition comprising:

a) at least 0.0001 weight percent of said perhydrolase of Claim 1;

b) a molecule comprising an ester moiety; and

c) optionally, an adjunct ingredient.

135. A cleaning composition comprising:

a) at least 0.0001 weight percent of said perhydrolase of Claim 1;

b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, said peroxygen source being selected from the group consisting of:

i. a per-salt;
ii. an organic peroxyacid;
iii. urea hydrogen peroxide;

iv. a carbohydrate and carbohydrate oxidase mixture, and
v. mixtures thereof;

c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety; and

d) optionally, an adjunct ingredient.

136. A cleaning composition comprising:

a) from about 0.0001 to about 1 weight percent of said perhydrolase of Claim 8;

b) a material selected from the group consisting of a peroxygen source, hydrogen peroxide and mixtures thereof, said peroxygen source being selected from the group consisting of:

vi. a per-salt
vii. an organic peroxyacid;
viii. urea hydrogen peroxide;
ix. a carbohydrate and carbohydrate oxidase mixture, and
x. mixtures thereof;

c) from about 0.01 to about 50 weight percent of a molecule comprising an ester moiety

d) optionally, an adjunct ingredient.

137. A cleaning composition according to Claim 136, said composition comprising an adjunct ingredient.

138. A cleaning composition according to Claim 137, wherein said adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme

stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids, pigments and mixtures thereof.

139. A cleaning composition according to Claim 137, wherein:

a) said perhydrolase exhibits a perhydrolysis to hydrolysis molar ratio that is greater than about 0.1;

b) said per-salt is selected from the group consisting of alkalimetal perborate, alkalimetal percarbonate, alkalimetal perphosphates, alkalimetal persulphates and mixtures thereof;

c) said carbohydrate is selected from the group consisting of mono- carbohydrates, di- carbohydrates, tri- carbohydrates, oligo- carbohydrates and mixtures thereof;

d) said carbohydrate oxidase is selected from the group consisting of aldose oxidase (IUPAC classification EC1.1.3.9), galactose oxidase (IUPAC classification EC1.1.3.9), cellobiose oxidase (IUPAC classification EC1.1.3.25), pyranose oxidase (IUPAC classification EC1.1.3.10), sorbose oxidase (IUPAC classification EC1.1.3.11) hexose oxidase (IUPAC classification EC1.1.3.5). glucose oxidase (IUPAC classification EC1.1.3.4) and mixtures thereof; and

e) said molecule comprising an ester moiety has the formula:

$$R^1O_x\,[(R^2)_m\,(R^3)_n]_p$$

(i) wherein R^1 is a moiety selected from the group consisting of H, substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;

(ii) each R^2 is an alkoxylate moiety;

(iii) R^3 is an ester-forming moiety having the formula: R^4CO- wherein R^4 is H, alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl,;

(iv) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1;

(v) p is an integer that is equal to or less than x;

(vi) m is an integer from 0 to 50; and

(vii) n is at least 1

140. The cleaning composition of Claim 139, wherein:

a) R^1 is an C_2-C_{32} substituted or unsubstituted alkyl or heteroalkyl moiety;

b) each R^2 is independently an ethoxylate or propoxylate moiety; and

c) m is an integer from 1 to 12.

141. The cleaning composition of Claim 140, wherein R^3 is an ester-forming moiety having the formula: R^4CO- wherein R^4 is:

a) a substituted or unsubstituted alkyl, alkenyl or alkynyl moiety comprising from 1 to 22 carbon atoms; or

b) a substituted or unsubstituted aryl, alkylaryl, alkylheteroaryl or heteroaryl moiety comprising from 4 to 22 carbon atoms.

142. The cleaning composition of Claim 137, wherein the molecule comprising the ester moiety has the formula:

$$R^1O_x[(R^2)_m(R^3)_n]_p$$

wherein:

a) R^1 is H or a moiety that comprises a primary, secondary, tertiary or quaternary amine moiety, said R^1 moiety that comprises an amine moiety being selected from the group consisting of substituted or unsubstituted alkyl, heteroalkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;

b) each R^2 is an alkoxylate moiety;

c) R^3 is an ester-forming moiety having the formula: R^4CO- wherein R^4 may be H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkylaryl, alkylheteroaryl, and heteroaryl;

d) x is 1 when R^1 is H; when R^1 is not H, x is an integer that is equal to or less than the number of carbons in R^1;

e) p is an integer that is equal to or less than x

f) m is an integer from 0 to 12; and

g) n is at least 1.

143. The cleaning composition of Claim 136, wherein said molecule comprising an ester moiety has a weight average molecular weight of less than 600,000 Daltons.

144. The cleaning composition of Claim 143, wherein said adjunct ingredient is selected from the group consisting of surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing

aids, pigments and mixtures thereof.

145. A method of cleaning, said method comprising the steps of:

a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 136 and/or a composition comprising the cleaning composition of Claim 136; and

b) optionally washing and/or rinsing said surface or material.

146. A method of cleaning, said method comprising the steps of:

a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 137 and/or a composition comprising the cleaning composition of Claim 137; and

b) optionally washing and/or rinsing said surface or material.

147. A bleaching composition comprising the perhydrolase of Claim 1.

148. The bleaching composition of Claim 147, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

149. A bleaching composition comprising the perhydrolase variant of Claim 8.

150. The bleaching composition of Claim 149, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases,

endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

151. A bleaching composition comprising the perhydrolase variant of Claim 113.

152. The bleaching composition of Claim 151, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

153. A bleaching composition comprising the perhydrolase variant of Claim 132.

154. The bleaching composition of Claim 153, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

155. A bleaching composition comprising the perhydrolase of Claim 3.

156. The bleaching composition of Claim 155, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall

degrading enzymes, hemicellulases, and cellulases.

157. A disinfecting composition comprising the perhydrolase of Claim 1.

158. The disinfecting composition of Claim 157, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

159. A disinfecting composition comprising the perhydrolase variant of Claim 8.

160. The disinfecting composition of Claim 159, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

161. A disinfecting composition comprising the perhydrolase variant of Claim 113.

162. The disinfecting composition of Claim 161, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

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163. A disinfecting composition comprising the perhydrolase variant of Claim 132.

164. The disinfecting composition of Claim 163, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

165. A disinfecting composition comprising the perhydrolase variant of Claim 3.

166. The disinfecting composition of Claim 165, further comprising at least one additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, endoglycosidases, lysozyme, bacterial cell wall degrading enzymes, fungal cell wall degrading enzymes, hemicellulases, and cellulases.

FIGURE 1

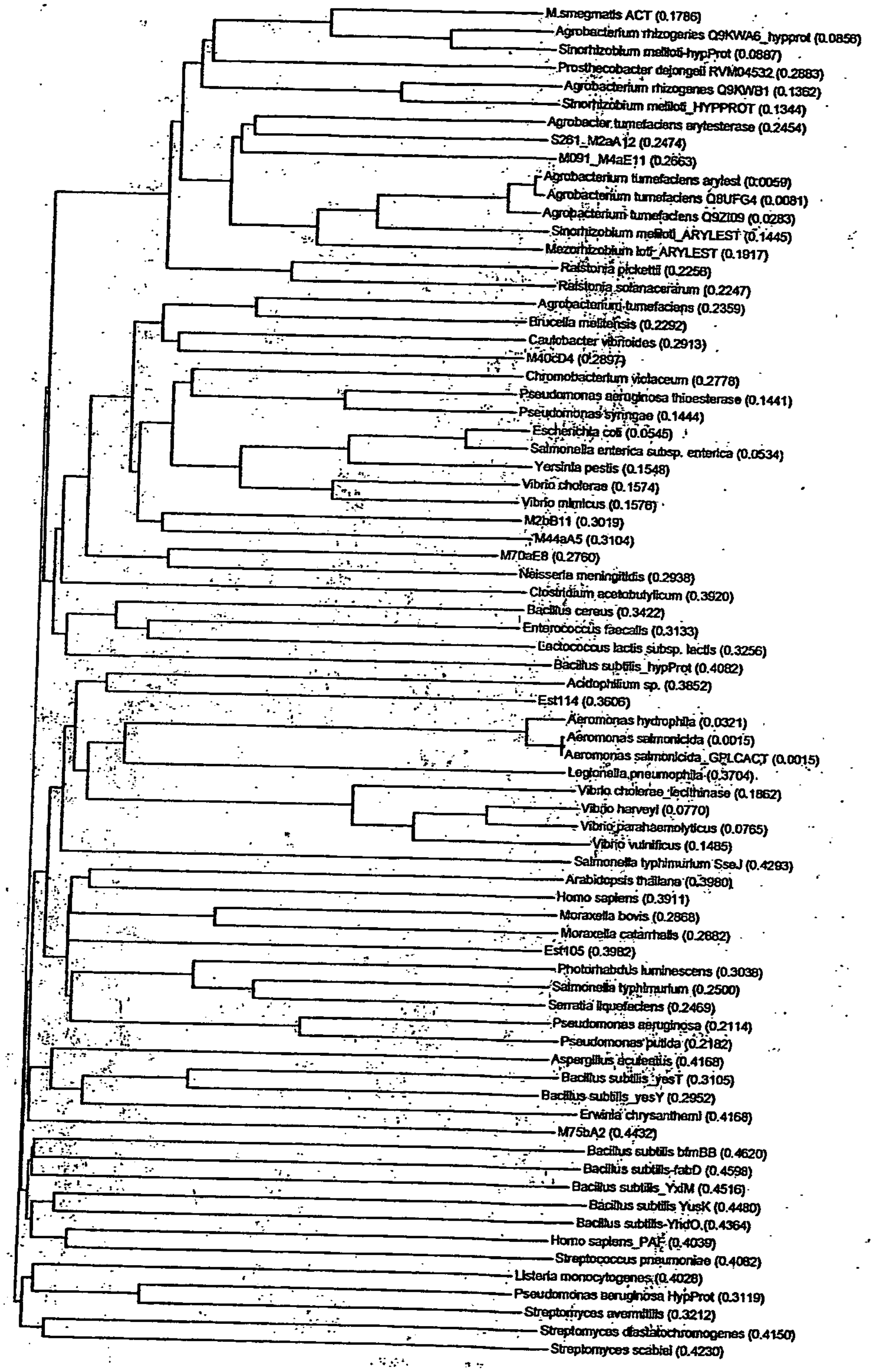

M.smegmatis ACT (0.1786)
Agrobacterium rhizogenes Q9KWA6_hypprot (0.0856)
Sinorhizobium meliloti-hypProt (0.0887)
Prosthecobacter dejongeii RVM04532 (0.2883)
Agrobacterium rhizogenes Q9KWB1 (0.1362)
Sinorhizobium meliloti_HYPPROT (0.1344)
Agrobacter tumefaciens arylesterase (0.2454)
S261_M2aA12 (0.2474)
M091_M4aE11 (0.2663)
Agrobacterium tumefaciens arylest (0.0059)
Agrobacterium tumefaciens Q8UFG4 (0.0081)
Agrobacterium tumefaciens Q9ZI09 (0.0283)
Sinorhizobium meliloti_ARYLEST (0.1445)
Mezorhizobium loti_ARYLEST (0.1917)
Ralstonia pickettii (0.2258)
Ralstonia solanacerarum (0.2247)
Agrobacterium tumefaciens (0.2359)
Brucella melitensis (0.2292)
Caulobacter vibrioides (0.2913)
M40cD4 (0.2897)
Chromobacterium violaceum (0.2778)
Pseudomonas aeruginosa thioesterase (0.1441)
Pseudomonas syringae (0.1444)
Escherichia coli (0.0545)
Salmonella enterica subsp. enterica (0.0534)
Yersinia pestis (0.1548)
Vibrio choleras (0.1574)
Vibrio mimicus (0.1576)
M2bB11 (0.3019)
M44aA5 (0.3104)
M70aE8 (0.2760)
Neisseria meningitidis (0.2938)
Clostridium acetobutylicum (0.3920)
Bacillus cereus (0.3422)
Enterococcus faecalis (0.3133)
Lactococcus lactis subsp. lactis (0.3256)
Bacillus subtilis_hypProt (0.4082)
Acidophilium sp. (0.3852)
Est114 (0.3606)
Aeromonas hydrophila (0.0321)
Aeromonas salmonicida (0.0015)
Aeromonas salmonicida_GPLCACT (0.0015)
Legionella pneumophila (0.3704).
Vibrio cholerae_lecithinase (0.1862)
Vibrio harveyi (0.0770)
Vibrio parahaemolyticus (0.0765)
Vibrio vulnificus (0.1485)
Salmonella typhimurium SseJ (0.4293)
Arabidopsis thaliana (0.3980)
Homo sapiens (0.3911)
Moraxella bovis (0.2868)
Moraxella catarrhalis (0.2682)
Est105 (0.3982)
Photorhabdus luminescens (0.3038)
Salmonella typhimurium (0.2500)
Serratia liquefaciens (0.2469)
Pseudomonas aeruginosa (0.2114)
Pseudomonas putida (0.2182)
Aspergillus aculeatus (0.4168)
Bacillus subtilis_yesT (0.3105)
Bacillus subtilis_yesY (0.2952)
Erwinia chrysanthemi (0.4168)
M75bA2 (0.4432)
Bacillus subtilis bfmBB (0.4620)
Bacillus subtilis-fabD (0.4598)
Bacillus subtilis_YxiM (0.4516)
Bacillus subtilis YusK (0.4480)
Bacillus subtilis-YhdO (0.4364)
Homo sapiens_PAF (0.4039)
Streptococcus pneumoniae (0.4082)
Listeria monocytogenes (0.4028)
Pseudomonas aeruginosa HypProt (0.3119)
Streptomyces avermitilis (0.3212)
Streptomyces diastatochromogenes (0.4150)
Streptomyces scabiei (0.4230)

FIGURE 2

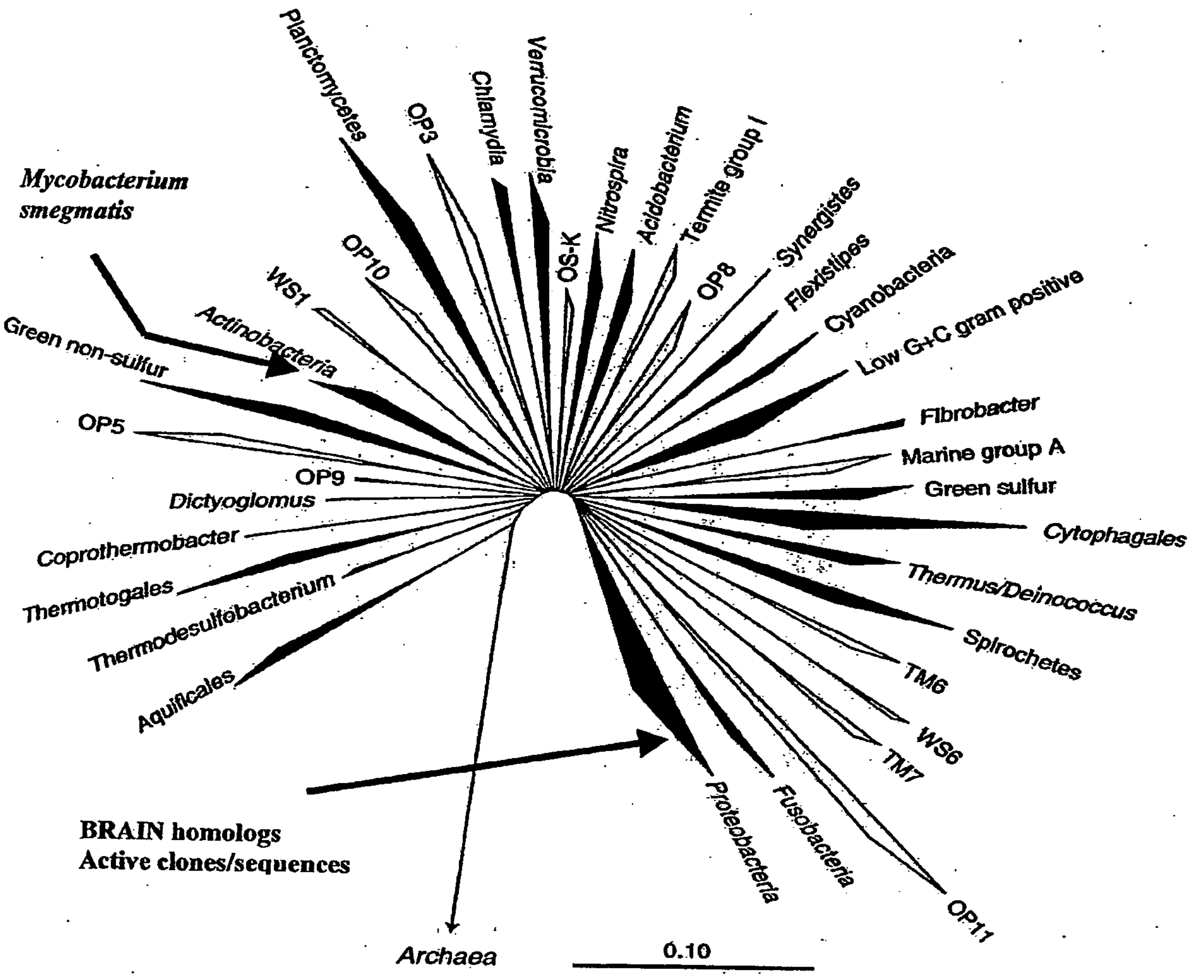

Mycobacterium smegmatis
Planctomycetes
OP3
Chlamydia
Verrucomicrobia
OS-K
Nitrospira
Acidobacterium
Termite group I
OP8
Synergistes
Flexistipes
Cyanobacteria
Low G+C gram positive
Fibrobacter
Marine group A
Green sulfur
Cytophagales
Thermus/Deinococcus
Spirochetes
TM6
WS6
TM7
OP11
Fusobacteria
Proteobacteria
OP10
WS1
Actinobacteria
Green non-sulfur
OP5
OP9
Dictyoglomus
Coprothermobacter
Thermotogales
Thermodesulfobacterium
Aquificales
BRAIN homologs
Active clones/sequences
Archaea
0.10

FIGURE 3

Chymotrypsin --His57—Asp102—Ser195

Subtilisin --Asp32—His64—Ser215

α/β Hydrolase --Ser126—Asp176—His206--

Perhydrolase –Ser11—Asp192—His195---

FIGURE 4

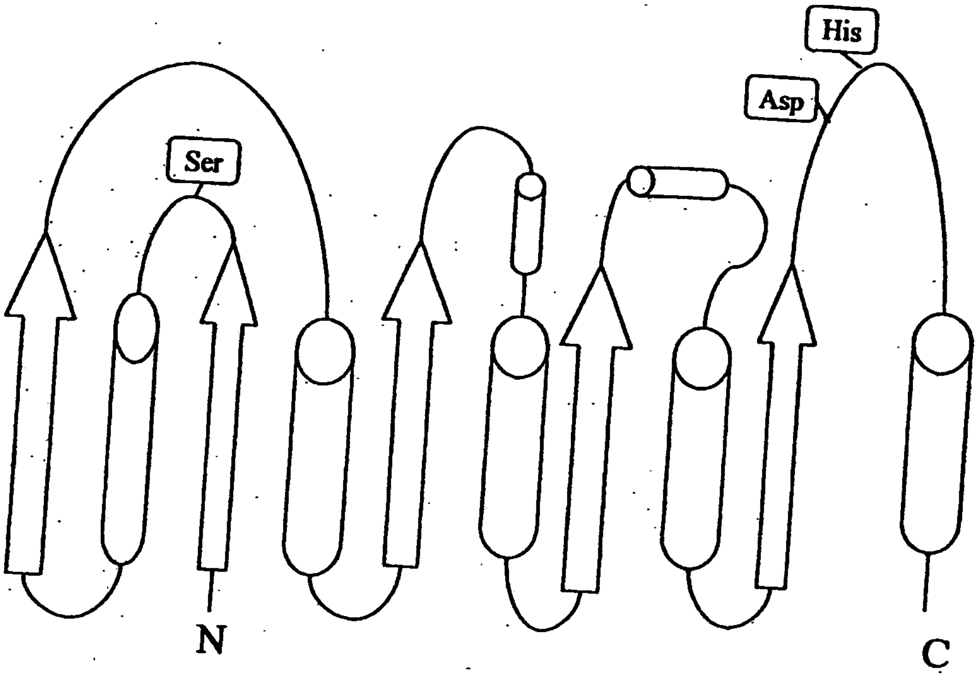


FIGURE 5

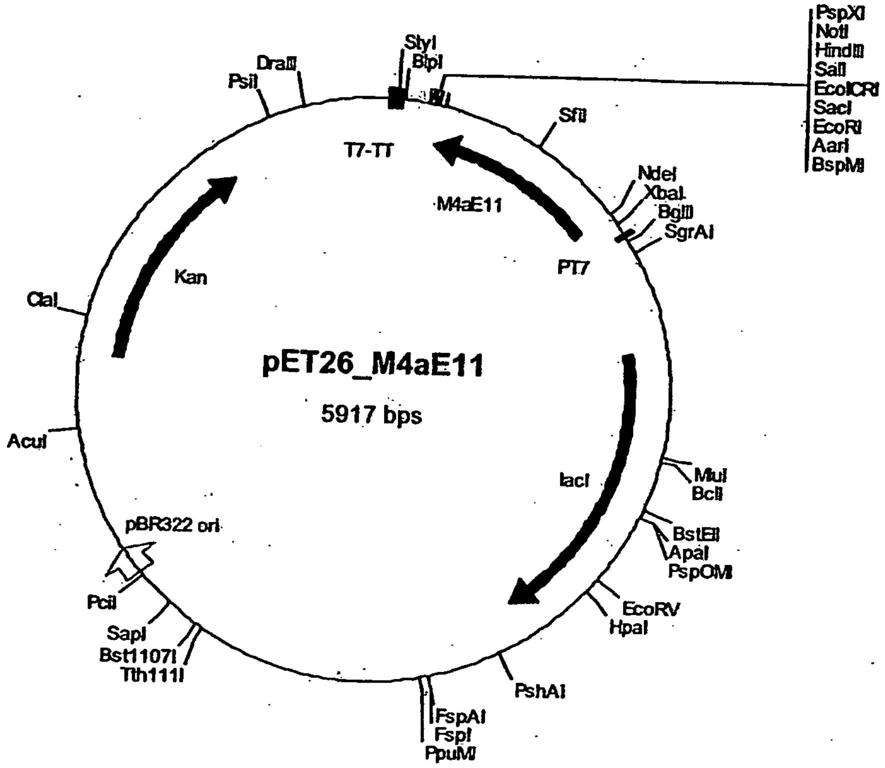

PspXI
NotI
HindIII
SalI
EcoICRI
SacI
EcoRI
AarI
BspMI
StyI
BlpI
DraIII
PsiI
SfiI
NdeI
XbaI
BglII
SgrAI
T7-TT
M4aE11
PT7
Kan
ClaI
pET26_M4aE11
5917 bps
AcuI
lacI
MluI
BclI
BstEII
ApaI
PspOMI
pBR322 ori
PciI
SapI
Bst1107I
Tth111I
EcoRV
HpaI
PshAI
FspAI
FspI
PpuMI

FIGURE 6

	Volume (ml)	Protein (mg/ml)	Total Protein (mg)	pNB Activity d410/min/ml	SpAc d410/min/mg	Total activity d410	Recovery (%)	Purification (fold)
Crude extract	128	7.5	960	3840	512	491520	100	1.0
55C 10min	118	5.4	637	3760	696	443680	90	1.4
Phenyl seph	241	0.37	89	1700	4595	409700	83	9.0
HQ Anion	0.74	22.7	17	250000	11013	185000	38	21.5

FIGURE 7

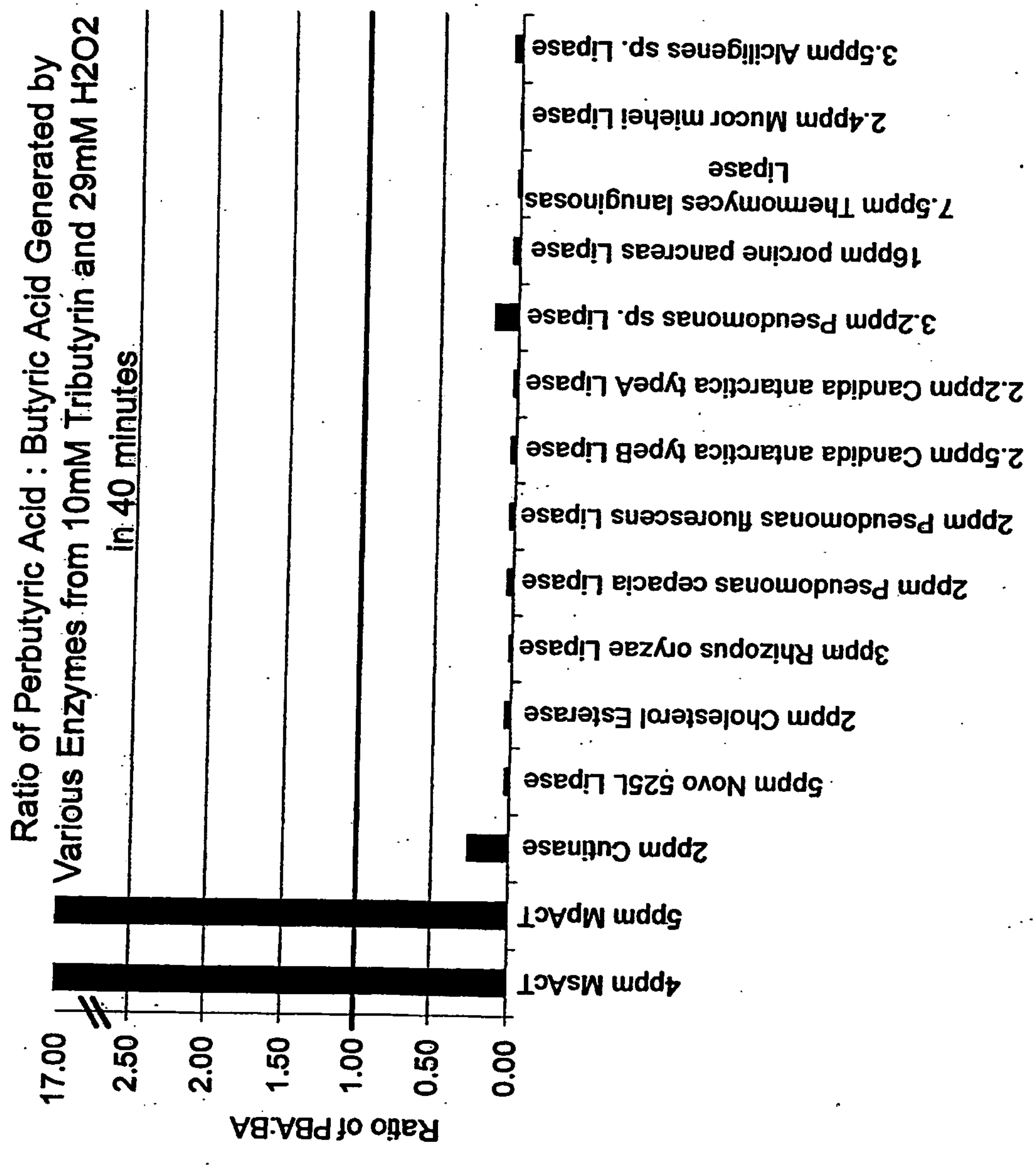
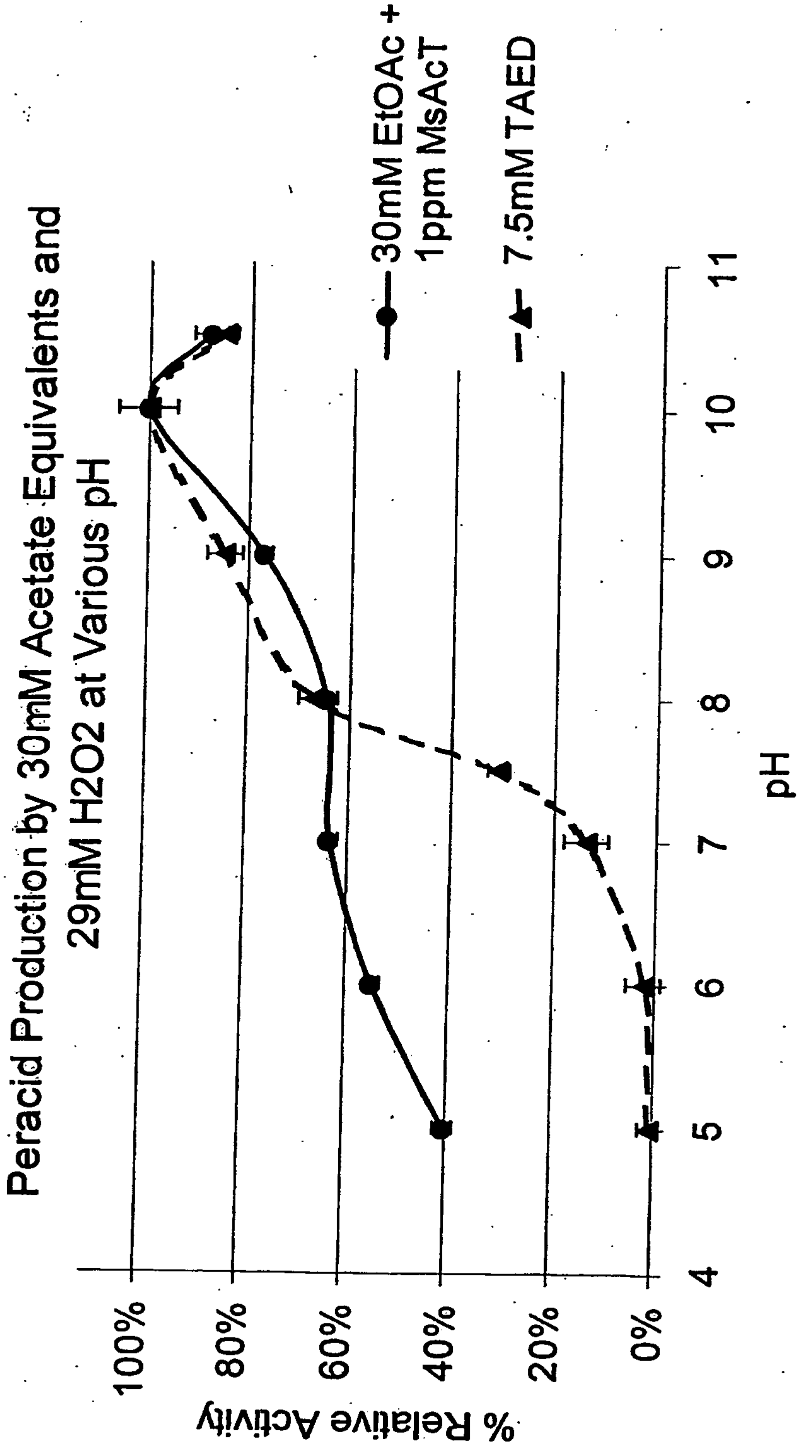

Ratio of Perbutyric Acid : Butyric Acid Generated by Various Enzymes from 10mM Tributyrin and 29mM H2O2 in 40 minutes
Ratio of PBA:BA
17.00
2.50
2.00
1.50
1.00
0.50
0.00
4ppm MsAcT
5ppm MpAcT
2ppm Cutinase
5ppm Novo 525L Lipase
2ppm Cholesterol Esterase
3ppm Rhizopus oryzae Lipase
2ppm Pseudomonas cepacia Lipase
2ppm Pseudomonas fluorescens Lipase
2.5ppm Candida antarctica typeB Lipase
2.2ppm Candida antarctica typeA Lipase
3.2ppm Pseudomonas sp. Lipase
16ppm porcine pancreas Lipase
7.5ppm Thermomyces lanuginosas Lipase
2.4ppm Mucor miehei Lipase
3.5ppm Alcligenes sp. Lipase

FIGURE 8



Peracid Production by 30mM Acetate Equivalents and 29mM H2O2 at Various pH

FIGURE 9

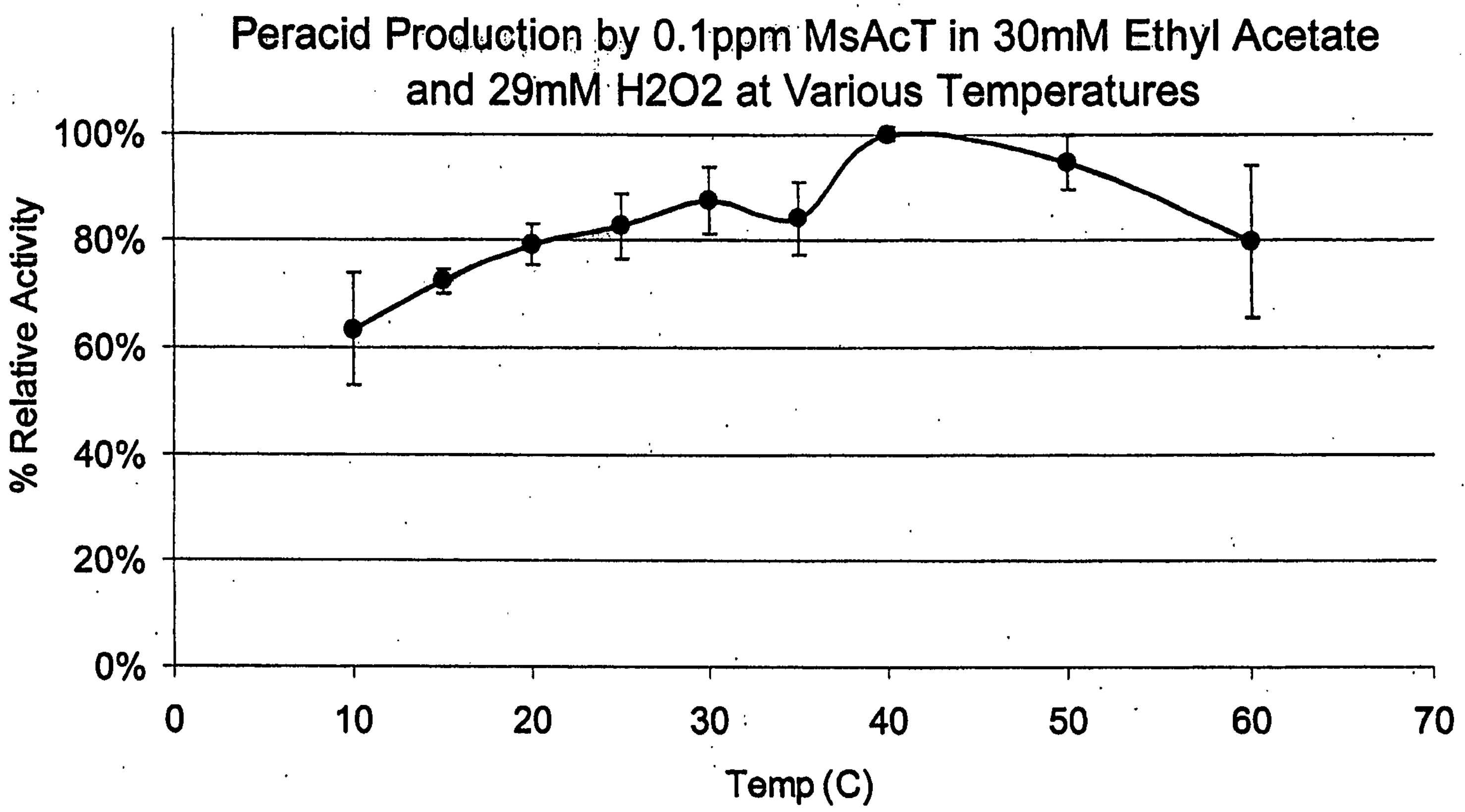

Peracid Production by 0.1ppm MsAcT in 30mM Ethyl Acetate and 29mM H2O2 at Various Temperatures
% Relative Activity
100%
80%
60%
40%
20%
0%
0
10
20
30
40
50
60
70
Temp (C)

FIGURE 10

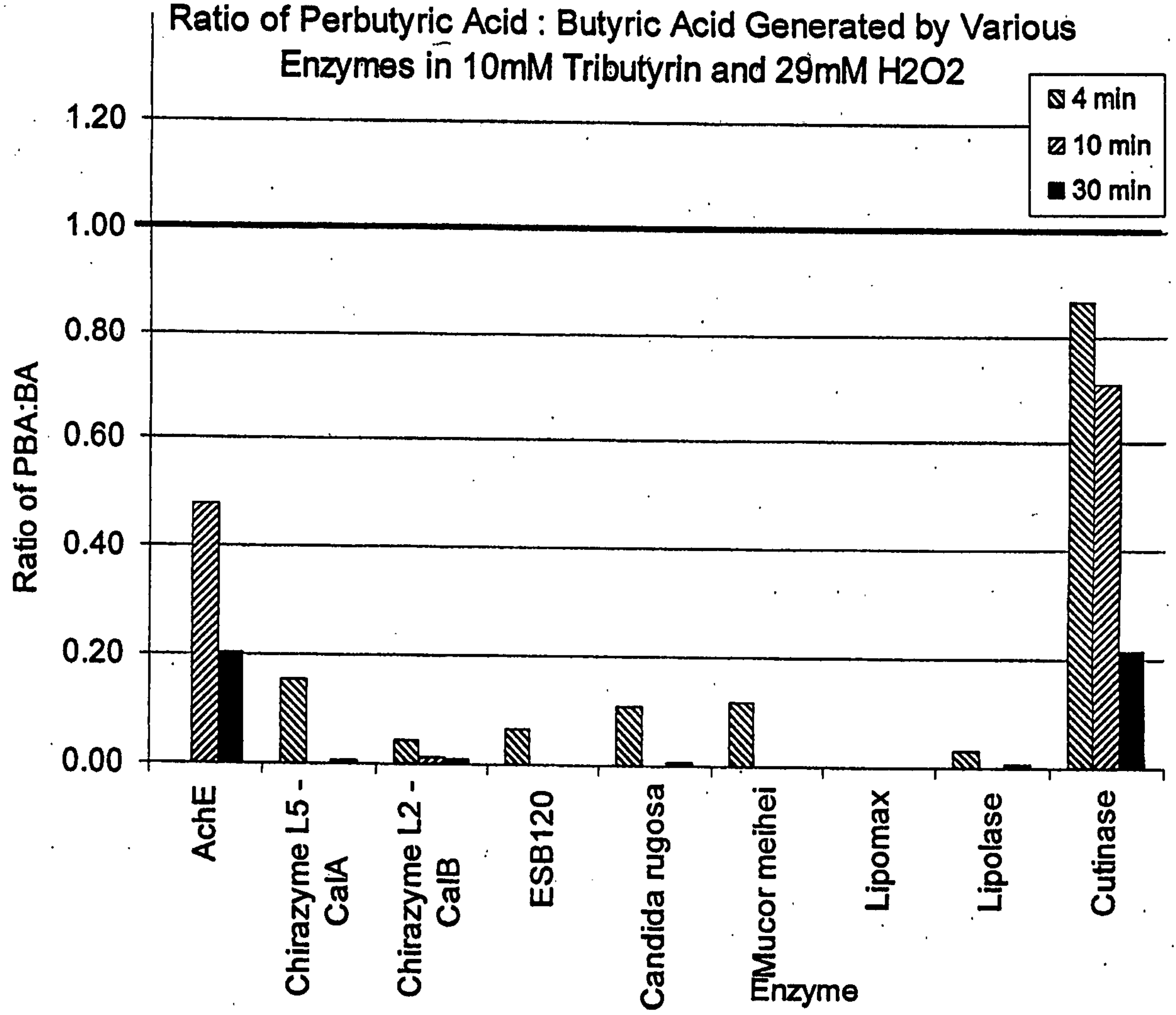
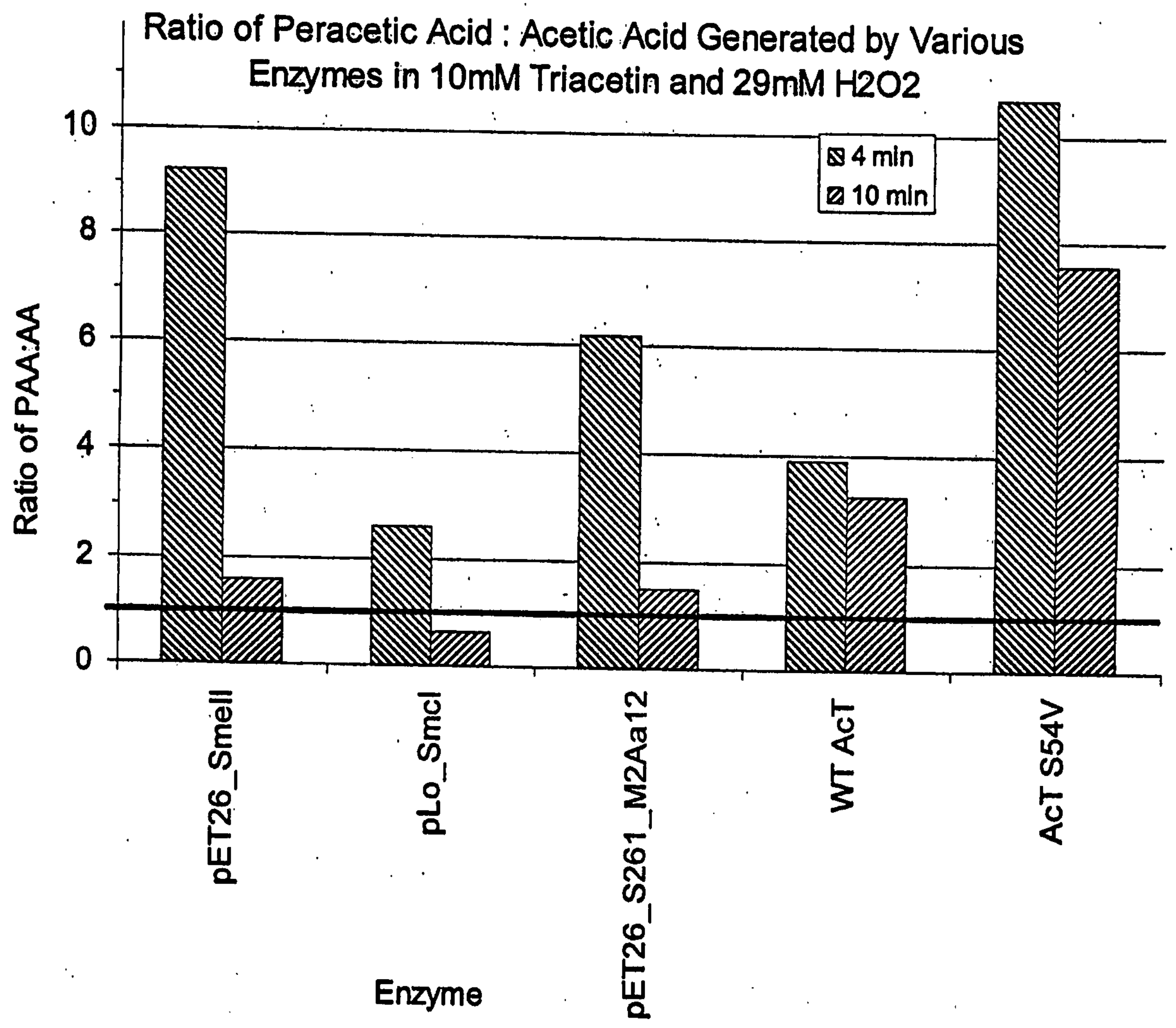

Ratio of Perbutyric Acid : Butyric Acid Generated by Various Enzymes in 10mM Tributyrin and 29mM H2O2
▧ 4 min
▨ 10 min
■ 30 min
Ratio of PBA:BA
1.20
1.00
0.80
0.60
0.40
0.20
0.00
AchE
Chirazyme L5 - CalA
Chirazyme L2 - CalB
ESB120
Candida rugosa
Mucor meihei
Lipomax
Lipolase
Cutinase
Enzyme

FIGURE 11



Ratio of Peracetic Acid : Acetic Acid Generated by Various Enzymes in 10mM Triacetin and 29mM H2O2

FIGURE 12

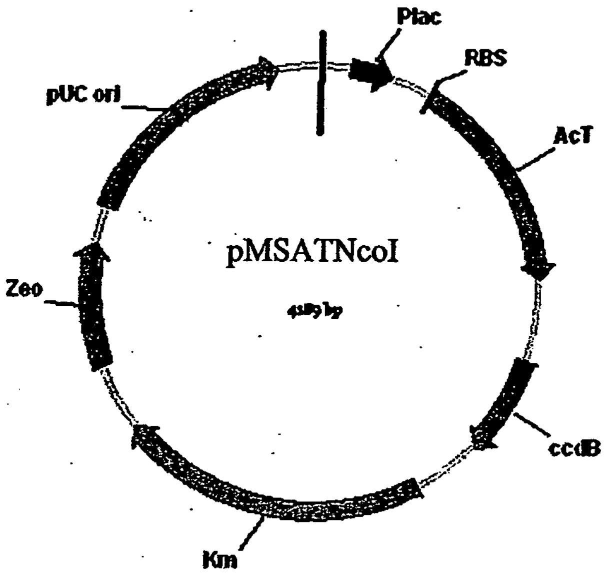


FIGURE 13

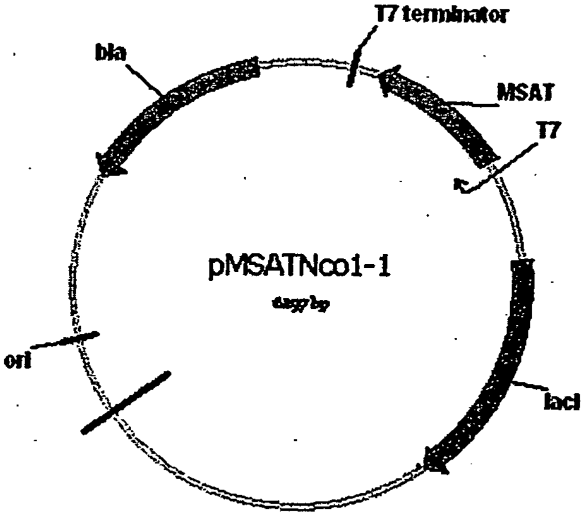


FIGURE 14

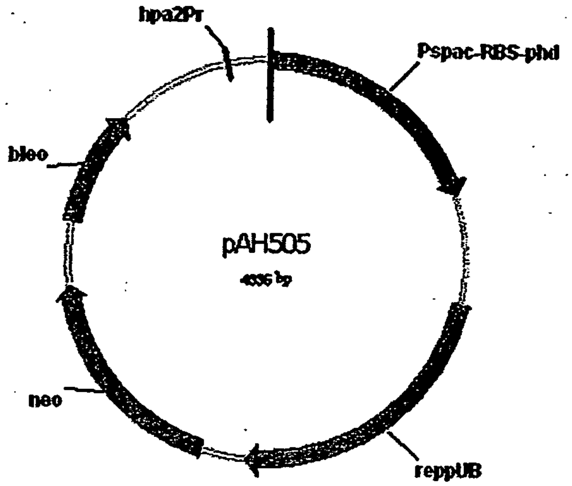


FIGURE 15

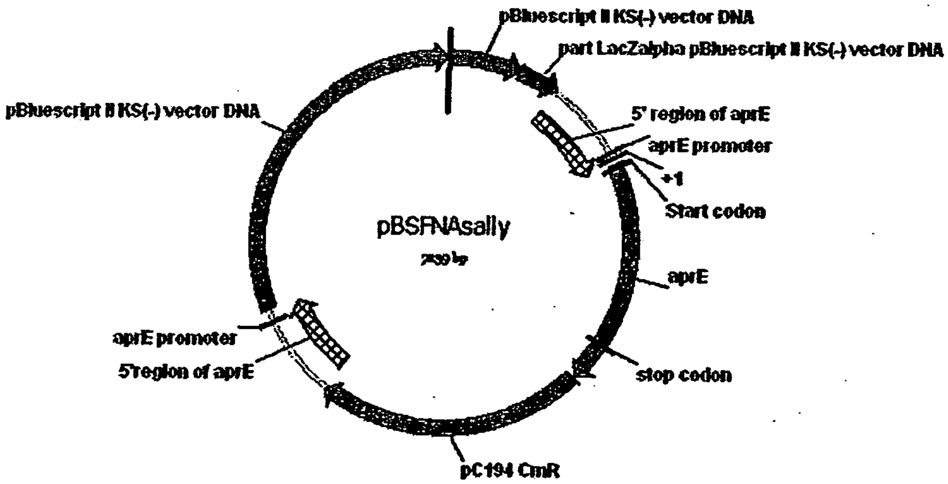


FIGURE 16

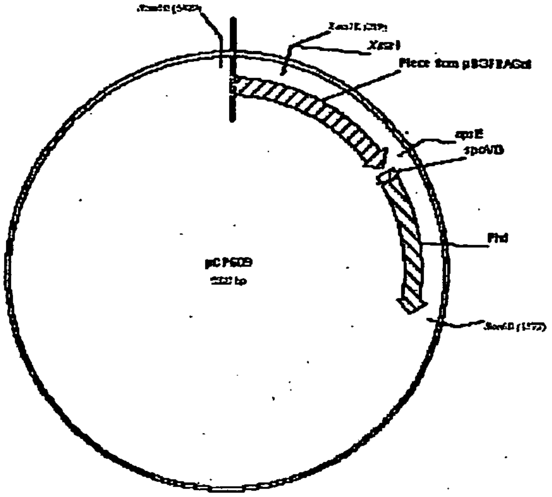


FIGURE 17

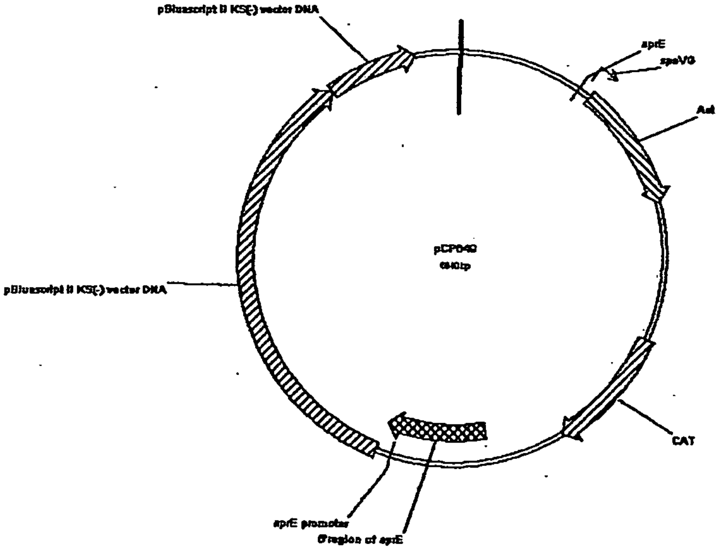


FIGURE 18

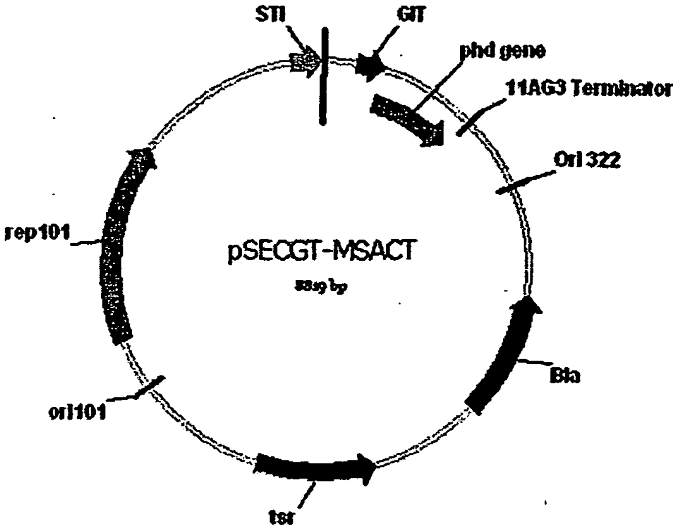


FIGURE 19

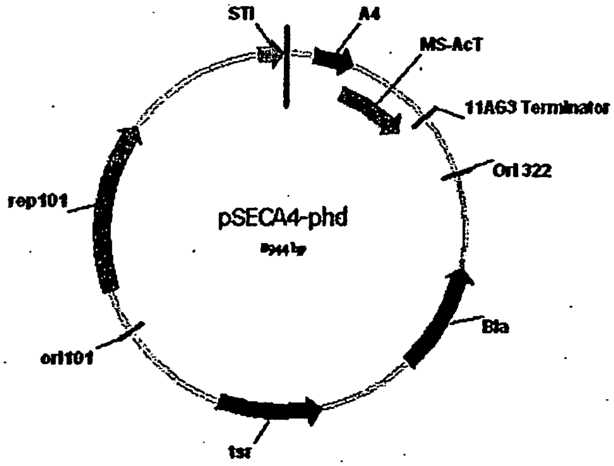


FIGURE 20

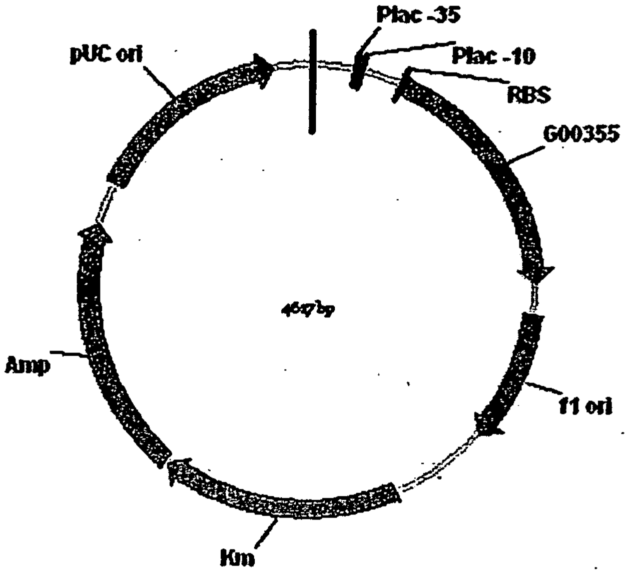


FIGURE 21

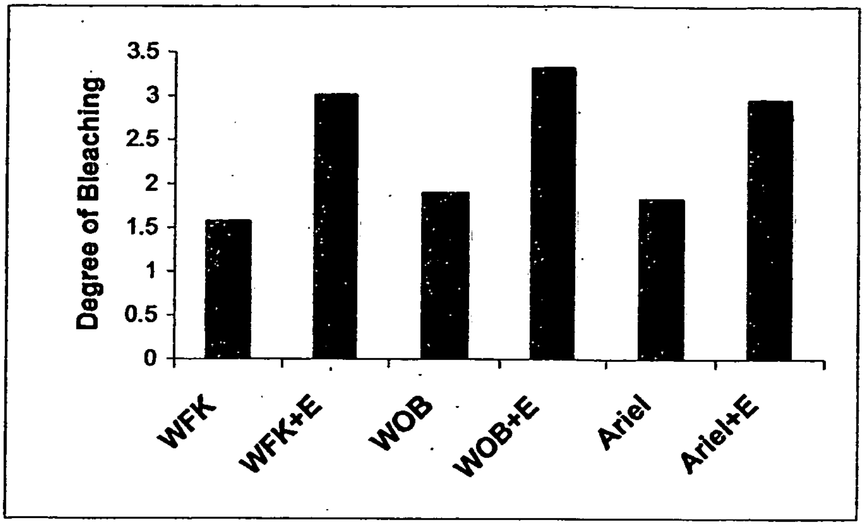


FIGURE 22

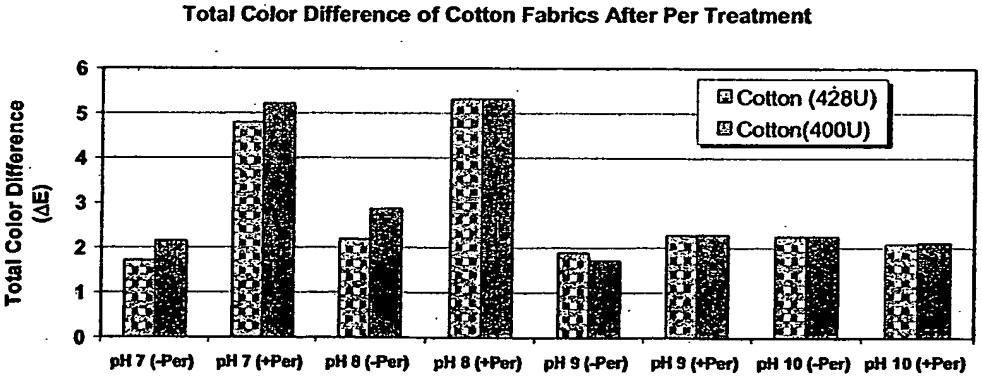
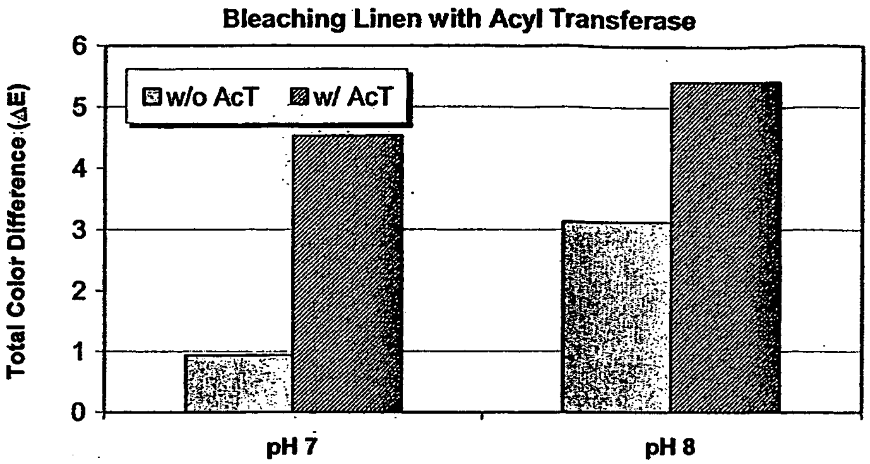


FIGURE 23


Bleaching Linen with Acyl Transferase
Total Color Difference (ΔE)
0
1
2
3
4
5
6
w/o AcT
w/ AcT
pH 7
pH 8